AUTHORIZED ENGLISH EDITION

TUBERCLE BACILLUS INFECTION AND TUBERCULOSIS IN MAN AND ANIMALS

PROCESSES OF INFECTION AND RESISTANCE
A BIOLOGICAL AND EXPERIMENTAL STUDY

WITH THIRTY-ONE TEXT ILLUSTRATIONS AND TWENTY-FIVE COLORED PLATES

BY

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PREFACE

Since the publication in 1895 of I. Straus's splendid book,[1] an immense number of studies on *Tuberculosis* have appeared in all languages.

In writing this work, my object has been to sift out from the most important of these studies,—and also from my own research of several years,—the scientific principles on which to base, *in the present state of our knowledge*, the campaign against the most terrible of human infectious diseases.

I have intentionally omitted all discussion of doctrines or of theories, and have restricted the bibliographic indications to those which in my opinion are strictly necessary to any one seeking details of observations or experiments of which I state only the conclusions. This was indispensable if I were to hope to make clear the most important facts and the new ideas.

I have attempted above all to write a work for the biologist and the investigator. This book then is addressed to physicians and to veterinarians and at the same time to laboratory workers, and it is my hope that the latter will derive from it fruitful suggestions for their researches.

Many of my friends were formerly my pupils and have been associated with me in my work for almost twenty years. Two of them, alas, Lucien Bruyant, agrégé professor, and Léon Massol, agricultural engineer, have passed away, one of them a victim of the disease which we were studying together, the other a modest and glorious hero of the great war for the liberation of humanity.

To them first, and then to my dear co-workers Camille Guérin and Maurice Breton and all those best of comrades who have shared my laboratory life, I dedicate this book in token of my very deep affection and in memory of those days both joyous and sorrowful through which we have lived together.

Also to M. Millot, in charge of the course in animal painting at the Museum of Natural History, I owe the warmest thanks for the care with which he has reproduced from my photographs the greater part of the numerous colored plates of this book. M. Masson has seen fit to make of the French edition a veritable work of art.

A. CALMETTE.

[1] *La tuberculose et son bacille*, Paris, 1895, Rueff & Cie.

TRANSLATORS' PREFACE

Interest in the problem of tuberculosis has never been greater than today, when, in this country at least, the effort to combat it is apparently meeting with success. The casual observer might infer that the campaign is built upon a thoroughly solid foundation of knowledge and that our understanding of the disease is complete. On the contrary, numerous problems remain to be solved. Much of the pathology of the disease must be rewritten in the light of the phenomena of reinfection; our understanding of tuberculosis immunity is still chaotic; the cure continues so near and yet so far; and many there are who contend that reductions in mortality are deceptive and that the results do not justify the expenditures of money and effort.

At such a time appears *L'Infection bacillaire et la Tuberculose chez l'homme et chez les animaux*, by Professor Albert Calmette. The author is one who stands out among a small internationally-known group to whom tuberculosis has been a life-long study and upon whose continued contributions year after year much of the present conception of the disease has been established. Never, probably, in the rush of this very work and of his varied services, would time have been found for such an undertaking had not Professor Calmette been forced to undergo the hardship of an internment at Lille throughout the Great War. It was under these circumstances that most of his book was compiled.

Many who have studied this work in the orginal French have been struck by the need of bringing it before the English reading public. It is at once a resumé of the ripe experience of a brilliant scientist, and a summary of our present scientific knowledge. It is a study which can be read and reread, not only for its facts, but also for its wealth of suggestion. The book is full of stimulating ideas, and quite as much so where the author is on debatable ground.

The translators desire to gratefully acknowledge the encouragement received from Dr. Linsly R. Williams of the Commission for the Prevention of Tuberculosis in France, of the Rockefeller Foundation; also the very great assistance of Dr. E. F. Ducasse of Paris, who, through his thorough knowledge of both the French and English languages; was ever ready with indispensable aid.

April, 1922.

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INTRODUCTION

THE VIRUS OF TUBERCULOSIS

Some Pages of History—Bayle, Laennec, Villemin and Robert Koch

The origin of the virus of tuberculosis probably dates back to the days of long ago when men were beginning to live in compact social groups. Elliott Smith and Armand Ruffer, Foquet, Wood Jones and Derry,[1] through the study of Egyptian mummies, have disclosed how it wrought havoc among subjects of the Rameses and the Pharaohs. In ancient times, the *Veda* of India, the *Zend-Avesta*, sacred book of the Parsees, the writings of Hippocrates, those of Celsus, of Aretaeus of Cappadocia (70 B. C.) and of Avicenna abound in documents relative to the history of *phthisis*.

But the human disease, which was finally to receive this name was not really given a definite character until the end of the 18th century when two English physicians, Th. Reid[2] (1782) and Baillie[3] (1793), called attention for the first time to *granulations* and *tubercles* which increase in size and whose centers become purulent to the point of forming large abscesses in the lung substance.

A little later (1810). G. L. Bayle,[4] thought that he could differentiate the *military tubercle* encountered in certain cases of phthisis from some other cartilage-like granular forms which, in his opinion, produced *tuberculous phthisis*. To him belongs the great credit of being the first to point out that miliary tuberculosis is not a local lesion confined to the lung, but a general disease "probably identical with scrofula."

It remained for Laennec (1781–1826) to lay the real foundation

[1] Bull. Archeological Survey of Nubia; 1907; Bull. Soc. Archeol. d'Alexandrie, 1907–12.

[2] *Essay on the nature and cure of phthisis pulmonalis*. Lond., 1782, Cadell.

[3] *The morbid anatomy of some of the most important parts of the human body*. Lond., 1793, Johnson; French transl., Paris, 1803, Sampson.

[4] *Recherches sur la phthisie pulmonaire*. Paris, 1810, Gabon.

of our knowledge of the pathological anatomy of tuberculosis. This medical genius who at the age of 35, was himself to fall a victim to the terrible malady whose study had made him illustrious, demonstrated clearly the *oneness or unity of tuberculous matter,* at first grey and translucent (*gray granulation*), then yellow and opaque, then purulent.

Laennec said, "*Tuberculous matter* can develop in the lungs and other organs in two principal forms: as *isolated bodies* (granulation,

Fig. 1. Laennec

miliary tubercle, non-caseous tubercle, caseous tubercle, ulceration or cavity), and as an *infiltration.*" He used thus to differentiate the two chief anatomical types of tuberculosis which we to-day call *follicular* and *non-follicular.* Thanks to the method of mediate auscultation, of which he was the brilliant originator, he learned to detect the development of tubercles in the living subject. Humanity will be forever grateful to him for having thus created the first scientific means of diagnosing phthisis.

"There is perhaps no organ," wrote Laennec,[5] "which is immune

[5] *De l'auscultation médiate ou traité du diagnostic des maladies des poumons et du coeur.* Paris, 1819, Brosson & Chaude.

against the development of tubercles. I shall here indicate those in which I have found them, and approximately in the order of frequency: bronchial and mediastinal glands, cervical glands, mesenteric glands, glands of all other parts of the body . . . ., the surface of the peritoneum and of the pleura, where very many small tubercles are ordinarily to be found in the grey and translucent, or non-caseous stage . . ., the spleen . . ., the brain . . ., the bodies of the vertebrae or the spaces between their ligaments, the thick portion of the ribs, all other bones . . . Tubercles develop more rarely in voluntary muscles than in any other part of the body. Occasionally, but very seldom, tubercle formation is primary in the organs just enumerated, especially in the intestinal mucous membrane and lymphatic glands, *and the development of tubercles in the lungs is the result of a secondary extension.*"

The infectious nature of the disease therefore seemed obvious to Laennec. Furthermore he believed in the close relationship between tubercles in the lungs and tubercles in the glands, to which the name of scrofula is given, "*and whose softening, as is well known, is very often followed by complete cure.*"

A little later, Cruveilhier[6] was to go still further in this conception. To his mind, *tubercles of the lungs are really scrofula of the lungs.*

Laennec regards the tubercle as a small *tumor* and Virchow,[7] applying to his study the then new method of examination by the microscope, shows it to be made up of a mass of small round cells with their nuclei extending almost to the periphery as in the case of lymphoid cells of the glands or spleen. Thereafter he regards the tubercle as a lymphoid follicle, a *lymphoma,* whose evolution ends at times in caseation, again in calcification or fibrosis, or yet again with complete resorption resulting in *cure.* But, according to Virchow, *the caseous infiltrations of the lung* (caseous pneumonias or broncho-pneumonias) have nothing in common with the genuine tubercle, although they too produce *phthisis.* The latter may be due therefore either to an invasion of tubercles in the Laennec sense, or to a catarrhal or exudative inflammation bringing on obstruction of the bronchi and pulmonary alveoli.

This dualistic conception had gained many followers toward the middle of the last century. In France it was supported by Ch.

[6] Bull. Soc. Anatomique, 1826, p. 171.

[7] *Die krankhaften Geschwülste,* Berl., 1865.

Robin, Lorian and Empis, and Jaccoud, the latter bringing to it for a long period the weight of his great authority as a clinician. Herard and Cornil on the other hand opposed it on pathologico-anatomic grounds.

The triumph of Laennec's idea of the unity (Unicisme) of tubercle became certain only when Villemin[8] furnished experimental proof of the *inoculability of the tubercle* and of *caseous material.*

Fig. 2. Villemin

The date of this discovery (1865), contemporary with the celebrated work of Pasteur on so-called spontaneous generation and his first researches into silk worm diseases, marks the beginning of a brilliant era during which our knowledge of the etiology and the pathogenesis of tuberculosis was to make rapid and decisive progress.

The first report of Villemin, presented on the 5th of December, 1865,

[8] *Etudes sur la tuberculose, preuves rationelles et expérimentales de sa spécificité et de son inoculabilité.* Paris, 1868.

at the Academy of Medicine, told of his results in inoculating human tuberculous matter into rabbits. He drew the following conclusions:

"*Tuberculosis is a specific affection.* Its cause lies in an *inoculable* agent. It therefore belongs among the diseases of *virus origin* and should be classified by the side of syphilis, nearer however to glanders."

A few months later, checking himself always by the experimental method, he brought proof that the virus of *pommelière*[9] (tuberculosis) of cows produces in the rabbit a disease identical with that which develops when the latter animal is inoculated with the virus of human phthisis. Furthermore he showed that this virus is inoculable not only into rabbits but also into guinea pigs, and with more difficulty into dogs and cats. He did not succeed in communicating it to the sheep. Hens and pigeons were found equally refractory.

During the years which followed, the facts announced by Villemin provoked the most violent controversies on all sides, and particularly at the Academy of Medicine at Paris. Colin,[10] Chauffard, Piorry, Pidoux, vainly attempted to mitigate the effects of these discoveries which were tending to nothing less than the destruction of time honored doctrines. "Experiments on animals," cried out Pidoux, "give such and such results and you, instead of controlling them by clinical experiments and by all the accepted facts of human physiology construct upon them a general theory of human tuberculosis and all diseases! For it you upset all the ideas already acquired. We must accept over night that phthisis falls from the clouds and that in its pathogenesis, the subject himself, habitus, hygienic surroundings, heredity and diatheses are of no account; that all depends upon an impossible tuberculous virus originating without doubt in a tuberculous individual who had it from some other individual and so on to the first man, who however had it from nobody at all and must have created it himself out of nothing."

It was not long before the echo of such tirades was silenced in the brilliant proofs which came from all sides to confirm the researches of Villemin. Herard first, Guéneau de Mussy, Hardy, H. Bouley, then particularly Chauveau in France, Klebs, Cohnheim in Germany, Clark in England, all brought new facts which no one dared longer

[9] Formerly used because of the resemblance of some bovine tubercles to a small apple (pomme).

[10] Bull. Acad. méd., 1866, **32**, 897; 1868, **33**, 550; 595; 599; 603; 645.

dispute. In 1868, Chauveau[11] was able to write, "it is now proven that the identity of tuberculosis and the diseases recognized as of virus origin is so complete and so absolute that one must either grant this property of virus origin to tuberculosis or deny that such origin exists. The conclusions which Villemin has drawn from his inoculation results have therefore the value which he has attributed to them."

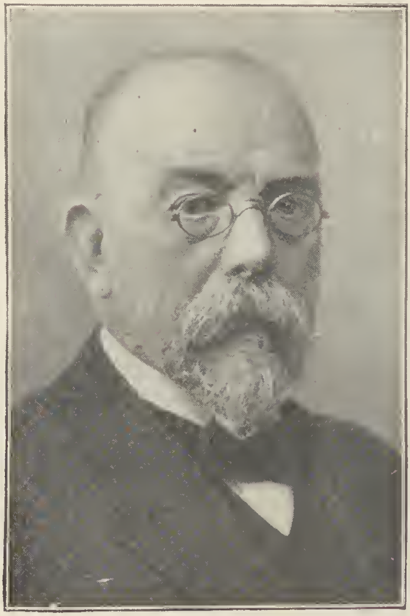


Fig. 3. Robert Koch

The case was settled. It remained to demonstrate the virulent agent by the methods orginated by Pasteur and perfected by Robert Koch for the isolation and for the study of pathogenic bacteria. The credit of discovering the *bacillus* was to fall to Robert Koch,[12] whose name remains gloriously associated with it.

The first publication on the discovery of the bacillus is a master-

[11] Gaz. hebdomadaire, 1868, p. 753.

[12] Berl. klin. Wchnschr., 1882, **8**, No. 15; Mitteil. a. d. k. Gsndhtsamte, Vol. **2**. The various studies published by Robert Koch on tuberculosis will be found assembled in two volumes edited by J. Schwalbe; *Gesammelte Werke von Robert Koch*, Leipzig, 1912, George Thieme.

piece still fresh despite the lapse of years. It established in a definite and an irrefutable manner the bacillary etiology of tuberculosis. It demonstrated that *the specific bacillus exists in the sputum of all phthisical subjects, in all tuberculous matter from man or animal, in scrofulous glands, in white swellings and in spontaneous as well as in experimental disease.* And Robert Koch furnished the proof that the microbe can be readily revealed wherever it exists, thanks to the special staining measures which Weigert had introduced into histological technique; that it can be cultivated on artificial media and that these cultures reproduce in susceptible animals the same lesions which characterize spontaneous tuberculosis.

"For the future," concluded Robert Koch, "in the campaign against the terrible scourge of tuberculosis, we no longer have to deal with something vague and undetermined; we are in the presence of a visible and tangible parasite, the conditions of whose existence we already know in part and can now study more closely. We know that the germ finds the conditions necessary for its existence only in the body of man and animals and that it cannot, like the bacillus of anthrax, develop in matter outside the animal economy: this fact is very consoling from the point of view of combatting the disease. From it we reason that we must devote ourselves, before anything else, to drying up the sources of infection. One of the sources, and certainly the chief one, is the sputum of tuberculous individuals, which must be disinfected and rendered innocuous. In this manner the largest element in the contagion of tuberculosis will be eliminated."

The publication of this memorable communication by Robert Koch, soon rendered more precise and complete by his further researches, was destined necessarily to have a most fortunate influence upon the development of opinion in favor of the experimental method. Thanks to the rapid progress of the latter, workers in all countries, clinicians, bacteriologists, hygienists, veterinarians, passionately attacked the study of tuberculosis, and so many are the works written on the subject during the last 30 years that their mere enumeration would fill several volumes. The reader will pardon me then if I cite only those to which he should refer in undertaking or controlling investigations.

The initial work by Villemin and the discovery by Koch are the scientific bases of our present knowledge of tuberculous infection. I shall not here go further into their story since all that the following chapters contain is but its development and amplification.

PART ONE

The Tubercle Bacillus and the Processes of Infection by It

CHAPTER I

MORPHOLOGY OF THE TUBERCLE BACILLUS

Methods of Examination, of Staining and of Differentiation

Tubercle bacilli are present in every tuberculous lesion. They are found in clumps in the center of miliary tubercles, in more or less considerable number in the pus of tuberculous abscesses, in the sputum of phthisical patients, in scrofulous glands and in some serous effusions (pleura, joints, peritoneum, etc.), in the skin alterations of lupus and at times in the circulating blood. But it is not always easy to demonstrate them, especially in old calcified or fibrotic lesions. *They can then be disclosed only by inoculating the ground up contents or walls of lesions into a susceptible animal such as the guinea pig.*

The bacilli are almost always enclosed within the cell elements; but when the latter die and disintegrate, the bacilli are set free and may then be excreted from the body by various normal or accidental excretory paths.

By direct microscopic examination, even with the highest magnification, only the very experienced observer can determine,—and then with uncertainty—whether he has to do with the tubercle bacillus or with other microbes which have the same appearance when in the fresh state. Fortunately the nature of the tubercle bacillus can be settled by taking advantage of its property of *fixing* certain anilin dyes in a manner to differentiate it from coexisting bacteria or cells in tuberculous lesions or products.

A. MORPHOLOGY

In the sputa of phthisical patients (*fig. 4*), *tubercle bacilli* have the form of slender non-motile rods, whose average length is from a quarter to half the diameter of a human red blood cell, 1.5 to 3.5 microns (at times 0.5 micron up to 8 microns according to Eastwood[1]) and whose thickness is about 0.3 micron. Usually they are found

[1] Rep. Royal Commission on Tuberculosis, 1907–1911.

singly or in groups of 2 or 3, or at times in small irregular clumps. Some are free, others are contained within polynuclear leucocytes. Only by staining can their form be well brought out, when they often appear slightly curved or beaded. Staining causes them to appear more thick, since the ectoplasm fixes the dye very intensely, although irregularly, with the result that some portions of the bacillus remain transparent while others become completely opaque. The transparent portions, from their resemblance to spores were regarded as such (G. Spengler[2]), but we know today that they are nothing but

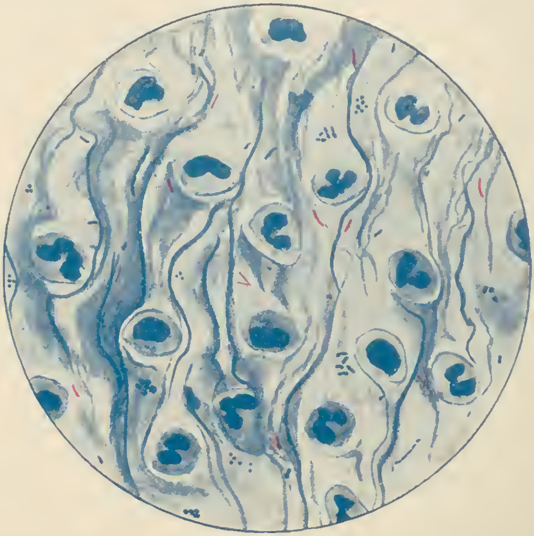


Fig. 4. Tubercle Bacilli in the Sputum of a Case of Phthisis

Stained with Ziehl and methylene blue. Imm. $\frac{1}{18}$; oc. comp. 6, Reichert.

small masses of protoplasmic substance having the characteristics of lipoids (the *Gram-positive granules* of Much).

The tubercle bacillus, at least during its parasitic existence, and also in cultures on artificial media, does not reproduce itself by sporulation but by elongation and transverse division of the rods. The ectoplasm is made up in part of fatty substance and a sort of wax, to which are due the double property of taking up certain anilin dyes only with difficulty when unheated and of retaining them, once taken up, in spite of the action decolorizing agents.

[2] Deutsch. med. Wchnschr., 1907, **33**, 337.

Cultures on artificial media always show a certain number of bacilli unusually long and thick and at times branched and with terminal clubbings, (Metchnikoff,[3] later I. Klein, Fischel, Hueppe). This applies especially to old cultures on solid media and more particularly to those of avian origin. Their appearance is then not unlike that of cultures from *actinomycosis* (*Actinomyces bovis*). Young cultures are both more readily stained and more readily decolorized (Nocard and Roux).

Microscopic study shows that the films formed on fluid media are composed of three sorts or elements dissimilar in their dye affinities (F. Bezançon and A. Philibert),[4] but we are totally ignorant of their respective rôles as regards virulence. These elements are:

1. A substance forming a sort of frame-work both membranous and fibrillar, *cyanophil and non-acid-fast;*

2. A *fuchsinophil* substance (bacilli properly speaking, more or less long, containing small chromophil violet granules (*acid-fast*);

3. *Gentianophil* granules, staining a violet black by the method of *Gram* and found either within the bacilli, or free and increasingly abundant in proportion to the age of the culture.

In tuberculous lesions it is impossible to distinguish the cyanophil substance; only bacillary forms and the chromophil bodies are to be seen in the sections.

In the case of certain rodents which are particularly resistant to tuberculosis, such as the *Gerbille* (*Meriones shawii*) which is found in the Sahara in Algeria and Tunis, tubercle bacilli, when introduced experimentally into the tissues, assume quite unusual forms. They have been described by Metchnikoff and will be studied later in this book (Chapter VI).

These forms, which J. E. Magroux calls *actinophytes*, are found occasionally in naturally developed tuberculosis, but they are very rare. Coppen Jones[5] has found them in the sputum of phthisical cases with cavity. Babès and Levaditi,[6] and Lubarsch obtained them by inoculating human or avian bacilli into the brain of the rabbit. Friedrich, and Otto Schulze[7] found them in the kidneys, in

[3] Virchow's Arch., 1888, **113**, 63.

[4] Bull. Soc. d'études scient. sur la tuberc., 1914, March 12, 32.

[5] Centralbl. f. Bakt., 1895, **17**, 1; 70.

[6] Arch. de méd. expér., 1897, **9**, 1041.

[7] Ztschr. f. Hyg., 1899, **31**, 153.

the lungs and in the brains of rabbits which had received inoculations of tubercle bacilli into the carotid artery.

Examination in the fresh state of very young cultures on fluid media shows that the bacilli possess a real motility and that they are provided with a variable number of flagella at each pole. Soon the flagella become entangled rendering the bacilli non-motile and apparently playing a capital rôle in the facility with which the organisms adhere to one another to form compact clumps. The fatty waxy ectoplasm keeps them agglutinated and floating in dry and wrinkled, more or less thick films, on the surface of the media.

The morphological characteristics of tubercle bacilli are greatly influenced by the chemical composition of the artificial media on which they are grown. They are at times shorter and more slender, or again longer and thicker. Similar variations occur also according as the bacilli are derived from old or recent lesions, from tuberculosis in active evolution or in process of cure, and furthermore according to the host which harbors them. Thus it is that bacilli of bovine origin are, generally speaking, shorter and thicker than those of human origin (*Plate I*).

B. TECHNIQUE OF STAINING METHODS

The technique for staining the tubercle bacillus has been the object of a large number of investigations.

The original method employed by Robert Koch was as follows: Slides bearing tuberculous material (either sputum or smears from organs) were left immersed for 24 hours in a staining bath composed of:

	cc.
Concentrated alcoholic solution of methylene blue............	1.0
Distilled water..	200.0
10 per cent potassium solution.............................	0.2

They were then washed in distilled water and left in a concentrated aqueous solution of Bismarck brown a few minutes, until they had taken on a frankly brownish tint. They were again washed in water, dried and mounted in Canada balsam. The tubercle bacilli retained their blue color and stood out quite clearly against the uniform brown color of the other elements on the slides.

But this method was unreliable. Ehrlich[8] soon greatly improved it by staining the bacillus with aniline methyl violet instead of alkaline methylene blue and next decolorizing the slides in a solution of nitric acid one part and water two parts. The tubercle bacilli then retain the violet color and appear almost black, while the other bacteria and cells take on a greyish tint.

But Ehrlich[9] himself soon showed that aniline fuchsin (chlorhydrate of rosanilin) gives even better results than methyl violet.

The staining methods of Koch-Ehrlich are no longer in use. The Ziehl-Neelsen[10] method is now preferred, and rightly so, as being quicker and more trustworthy.

The staining bath of Ziehl-Neelsen is prepared as follows:

One gram of fuchsin rubine is ground up in a mortar in 10 cc. of absolute alcohol. To this 5 gms. of white phenol crystals are

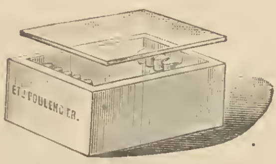


FIG. 5. STAINING JAR

added and then 60 cc. of *distilled water* in small quantities, with constant stirring, and the whole poured into a flask. The mortar is rinsed with 40 cc. of *distilled water*, which is also added to the flask. The solution is allowed to stand 24 hours and is then filtered.

The preparations of tissue smears or of sputum are immersed in this bath in a large Borrel tube or in any other suitable receptacle, which is then left for two hours in the incubator at 37°C. (*fig. 5*). Or again, if hurried, a few drops of the staining fluid may be poured directly on the slide, which is then heated in a Bunsen flame or over an alcohol lamp until the fluid steams. This heating is continued at least three minutes.

The slide is next washed an instant in cold water to remove the excess of stain, and a solution of nitric acid (one part of acid to three parts of distilled water), or of sulphuric acid (one part to four parts of water), or again a solution of acetic acid (one part of acid to two

[8] Deutsch. med. Wchnschr., 1882, **8**, 269.
[9] Charité Annalen, Berlin, vol. II, p. 123.
[10] Deutsch. med. Wchnschr., 1882, **8**, 451; 1883, **9**, 247.

parts of 95 per cent alcohol) is poured on. This is allowed to act for 20 seconds. The slides are washed in 60 per cent alcohol until the preparation is well decolorized, and are then rinsed in water.

Counterstaining is done with an aqueous solution of methylene blue, or better with the *blue of Kühne* applied for 20 seconds.

Methylene blue	1.5 gm.
Absolute alcohol	10.0 cc.
Phenol solution 5 per cent	100.0 cc.

Finally the slides are washed in running water for two or three seconds, then dried and examined with the oil immersion lens.

If the slide is to be preserved, the oil should be removed with xylol and the preparation dried. It can then later be re-stained if necessary, since the bacilli lose their color with time, especially after prolonged exposure to daylight.

By this method of Ziehl-Neelsen, the tubercle bacilli stand out sharply *red* against a *blue* background.

However, instead of employing a one-quarter strength solution of nitric acid or a one-fifth solution of sulphuric or acetic acid as a decolorizing agent after staining with carbol-fuchsin, it will be found preferable to use a 2 per cent solution of *aniline chlorhydrate* in water, as recommended by Kühne.[11] This reagent is much less harsh, and less destructive to the histological elements which accompany the tubercle bacillus in the preparations. It is allowed to act about 30 seconds. Decolorization is then completed in 95 per cent alcohol and the slide washed in water and counter-stained with methylene blue as already described.

A solution of *1 per cent sulphite of soda,* allowed to remain on the slide for one to two minutes, may also be used to decolorize.

Numerous other procedures have been proposed and various advantages claimed for them. I am describing only a few, stating at the outset that the method of Ziehl-Neelsen with the Kühne modification is infinitely superior to all the others (*see Chapter XXXIII*).

Method of Weichselbaum.[12]

Stain with hot *carbol-fuchsin* as in the Ziehl method;

[11] *Praktische Anleitung zum mikroskopischen Nachweis der Bakterien im tierischen Gewebe,* Leipz., 1888, Günther; Centralbl. f. Bakt., 1890, **8**, 293.

[12] Wien. med. Wchnschr., 1883, **33**, 63.

Wash in water; counterstain 30 seconds with a *saturated alcoholic* solution of methylene blue; wash in water.

Method of Rondelli and Buscalioni.[13]

Stain with carbol fuchsin according to Ziehl;

Decolorize 2 to 3 minutes with *Javel water.* This is prepared by dissolving 6 gms. of *calcium hypochlorite* in 60 cc. of water; and 12 gms. of *potassium carbonate* in 40 cc. of water, filtering separately and mixing before using.

The background of preparations decolorized with this fluid becomes brownish: it is therefore useless to counterstain.

Method of Fraenkel-Gabbet.[14]

Stain with the Ziehl's *carbol-fuchsin.*

Decolorize and counterstain simultaneously with a saturated solution of methylene blue in:

	cc.
Absolute alcohol	50
Sulphuric acid	25
Distilled water	100

Method of Müller.[15]

Stain with Ziehl's *carbol-fuchsin;*

Wash in water;

Decolorize in a *10 per cent solution of bicarbonate of soda in 70 per cent alcohol* at least 15 minutes, or 5 to 10 minutes in *hydrogen peroxide (oxygen 12 volumes) rendered alkaline with sodium hydroxide;*

Counterstain with *methylene blue.*

Method of Von Betegh.[16]

Pour upon the smears, previously fixed by heat, a few drops of *15 per cent nitric acid* and heat a few seconds, this serving as a mordant.

Wash, then stain a few minutes with a mixture of one to two drops of Loeffler's *alkaline methylene blue* and two or three drops of *carbol fuchsin;* heat once more. Wash in water.

Decolorize in *60 per cent alcohol* and counterstain for one to two minutes with *malachite green* (saturated aqueous solution); wash, dry and mount in balsam or cedar oil.

[13] Centralbl. f. Bakt., 1897, **21**, 70.

[14] Berl. klin. Wchnschr., 1884, **21**, 193; 214; Lancet 1887, i, 757.

[15] Centralbl. f. Bakt., 1901, **29**, 791.

[16] Ibid., 1908, **47**, 654; 1909, **49**, 461; 1909, **52**, 550.

The bacilli are colored red while the granules take on a dark blue tint against a green background.

Method of Herman[17] (of Mons).

Stain for one minute over a low Bunsen flame with a mixture of one part of a *3 per cent solution of crystal violet in absolute alcohol* (or in 95 per cent methyl alcohol) and three parts of a *1 per cent solution of ammonium carbonate* in distilled water.

Decolorize a few seconds in *10 per cent nitric acid,* then in *95 per cent alcohol,* to a pale blue color. Wash quickly in distilled water and counterstain either with a *1 per cent aqueous solution of eosin,* an *alcoholic solution of carmine,* with *Bismarck brown* or with a *1 per cent aqueous solution of safranin.*

The bacilli and the granules derived from them are stained an intense homogeneous blue, while the background is either red or brown according to the counterstain employed. This is a very good method, but a little longer and more complicated than that of Ziehl.

Methods of C. Spengler.[18] There are several of them. The last one proposed, which is in fact but a modification of the others, is as follows:

Stain with hot *carbol-fuchsin;*

Wash; leave in a *saturated alcoholic solution of picric acid* for two or three minutes.

Wash in 60 per cent alcohol and in 15 per cent nitric acid for 20 to 25 seconds.

Wash again in alcohol until completely decolorized. Wash in water.

Treat for the last time with the picric alcohol solution. The brilliant red bacilli contrast well against the yellow.

Method of Spengler, *modified by* P. Spehl.

Stain for 2 or 3 minutes with heat, or for 15 to 30 minutes cold in a freshly prepared mixture of 3 parts of Ziehl fuchsin solution and 2 parts of gentian violet;

Treat with picric alcohol (saturated solution of picric acid in water, 60 parts; alcohol, 40 parts) during one minute;

Alcohol 60 per cent strength;

Decolorize with weak nitric acid (1 part to 6 parts of water), then with 60 per cent alcohol;

[17] Ann. de l'Inst. Pasteur, 1889, **3**, 160; 1908, **22**, 92.

[18] Deutsch. med. Wchnschr., 1907, **33**, 337.

Stain for one minute in the picric alcohol;

Wash in water; dry.

The bacilli are red, the granules of Much are black, the background is of a pale yellow color.

Methods of Much.[19] This author too has proposed several methods, which are modifications of the *Gram stain.* The best one is this:

Stain for 24 to 48 hours in the following solution:

	cc.
Concentrated alcoholic solution of methyl violet BN	10
2 per cent solution of carbolic acid	100

Immerse 12 minutes in the *iodine-iodide solution of Gram-Lugol;*

Treat one minute with a *5 per cent solution of nitric acid;*

Treat 10 seconds with *3 per cent solution of hydrochloric acid;*

Wash in *acetone alcohol* (equal parts of each) until all color is eliminated;

Counterstain with *dilute fuchsin solution* or with *1 per cent aqueous solution of safranin* (5 to 10 seconds);

Wash in water; dry.

This method gives good results, but it is long and complicated and the preparations do not last as well as those stained by the Ziehl method. It brings out the lipoid granules and the degenerated bacilli. These granular forms pointed out by Much are not however to be regarded as special forms of tuberculous virus. R. Bittrolff and K. Momose[20] have demonstrated that they are also stainable by the Ziehl method.

A. Kirchenstein has modified Much's method as follows:

Stain the bacilli by the *picric acid* method of Spengler (differentiation with the picric alcohol solution is not indispensable);

Wash carefully;

Stain with *dahlia-violet* or with *methyl-violet* for two or three minutes, heating to the point of steaming;

Wash and decolorize in a *5 per cent solution of iodide of potassium in 80 per cent alcohol.* Decolorization takes place in 10 to 15 seconds. Successfully prepared slides should appear greyish blue or pale blue to the naked eye;

[19] Berlin. klin. Wchnschr., 1908, **45**, 691; Beitr. z. Klin. d. Tub. 1907, **8**, 85; 357.

[20] Veröffentl. d. R. Koch Stift. z. Bekampf. d. Tuberk., 1913, H. **4**, 18.

Wash for some time and dry. Prolonged washing is necessary if one wishes lasting preparations. Any traces of iodine not removed tend to favor decolorization.

By this method the granules are colored a blackish blue. Young bacilli may show a granule at each extremity. Fully developed bacilli show 5 to 9 of them. The envelope of the bacillus is barely visible.

Method of Gasis.

Gasis[21] was guided by the idea that the tubercle bacillus by virtue of its chemical composition is more resistant to alkalies than to acids. He used first as *mordant* a *solution of eosin* prepared as follows:

Eosin crystals	1 gm.
Absolute alcohol	5 cc.
Distilled water	95 cc.

This mixture is poured into a small Erlenmeyer flask to which is then added a quantity of *bichloride of mercury* the size of a lentil. The mixture is gently boiled, with shaking, until the sublimate is completely dissolved. The solution clears and throws down a precipitate. The supernatant fluid is applied hot to the preparation for two or three minutes as a mordant.

Wash in water and treat with an alkaline decolorizing reagent made up as follows:

Sodium hydrate	0.5 gm.
Potassium iodide	1.0 gm.
50 per cent alcohol	100.0 cc.

until the red color disappears and is replaced by a greenish tint.

Wash carefully in absolute alcohol to eliminate the decolorant; then rinse in water and counterstain cold for two or three seconds with an *acid solution of methylene blue* made up as follows:

Methylene blue crystals	1.0 gm.
Absolute alcohol	10.0 cc.
Hydrochloric acid	0.5 cc.
Distilled water	90.0 cc.

Wash in water and dry.

[21] Centralbl. f. Bakt., 1909, **50**, 111; Berl. klin. Wchnschr., 1909, **46**, 836; 1910, **47**, 1449.

This method gives very brilliant preparations. The bacilli have a beautiful red color against a blue background.

Finally I shall mention a technique described by Fontès (of Rio de Janeiro), which lends itself readily to the study of the chromatophil granules of tubercle bacilli.

Method of Fontès.[22]

Stain with *Ziehl fuchsin* for 2 minutes, heating to vaporization. Wash in water; stain with *carbol crystal violet* for 2 minutes. Cover the slide (without washing) with Gram's *iodine-iodide solution*, pouring off and renewing three times; treat with *acetone-alcohol* until completely decolorized; wash in water; counterstain rapidly with an *aqueous solution of methylene blue*. Dry and examine in immersion oil.

The bacillus, in cultures as well as in sputum, now appears made up of two parts: a protoplasmic portion stained red and granules stained a dark violet. The older the bacilli the more numerous are the granules (up to about 6). Young bacilli ordinarily show only a single central chromatophil granule.

I do not think it worth while to describe any other procedures such as those of Kronberger,[23] Yamamoto,[24] Giaconi,[25] and C. Biot,[26] etc. which have no advantages over those already given. As I said before, the method of Ziehl-Neelsen, properly employed, fills every need and still remains the best.[27]

C. CONCENTRATION (HOMOGÉNÉISATION) OF TUBERCULOUS MATERIAL

When tubercle bacilli are to be sought for in material where they are present in but small numbers,—and it is then that their discovery is of the more value in clearing up a diagnosis,—it is always advantageous to bring about their separation from other matter (such as masses of pus, cell detritus, etc.,) in which they may be scattered and often concealed.

[22] Centralbl. f. Bakt., 1909, **49**, 317.

[23] Beitr. z. klin. d. Tuberk., 1910, **16**, 157.

[24] Centralbl. f. Bakt., 1908, **47**, 570.

[25] Fortschr. f. Med., 1883, No. 5.

[26] Gaz. des hôp., 1914, **87**, 42.

[27] Critical summaries of the different methods of staining the tubercle bacillus will be found in the articles of Berger (Centralbl. f. Bakt., 1910, **53**, 174), Dold (Arb. a. d. k. Gsndtsamte, 1911, **36**, 433), and Böhm (Centralbl. f. Bakt., 1912, **62**, 497)

The methods of concentration enable us to do this very well.

They consist in the digestion of sputum or feces, for exnmple by various enzymes or by chemical solvents. The bacilli are not dissolved thanks to the waxy fatty capsule, and they can afterward be collected either by simple decantation, by centrifugation, or by causing the bacilli to adhere to particles of oil or similar substances.

Biedert,[28] then Muhlhauser and Czaplewski,[29] were the first to recommend shaking the sputum with 3 or 4 times its volume of a *0.2 per cent solution of sodium hydroxide.* The tube is stoppered with a rubber cork and shaken violently. The material is poured out into a conical jar, neutralized with a *5 per cent solution of acetic acid* with a few drops of phenolphthalein as indicator, and the sediment allowed to settle out; or else the material is centrifuged after the addition of two parts of 90 per cent alcohol for each part of the fluid. The sediment is then spread upon slides and stained by Ziehl.

Hammerl[30] treats the sputum with a mixture of equal parts of *sodium and ammonium hydroxide*, then shakes with a small quantity of *acetone* and centrifuges.

Spengler,[31] and Von Philipp prefer to digest the sputum 24 hours in the incubator at 37° with a small quantity of *pancreatin.*

Von Ellerman and Erlandsen[32] bring about auto-digestion by mixing the sputum with half its volume of *0.6 per cent solution of bicarbonate of soda* and incubating for 24 hours at 37°C. The mixture is to be centrifugated in a graduated tube. To one volume of the sediment are added *4 volumes of a 0.25 per cent solution of sodium hydroxide.* The mixture is heated to boiling, with careful shaking, and centrifugated a final time.

Bezançon and Philibert[33] attach great importance to the specific gravity of the liquid from which the bacilli are to be separated. They have worked on a method the express object of which is to lower this gravity. This technique is as follows:

A given volume of sputum is measured out in a graduated glass. Ten times this quantity of water is measured out in another graduated

[28] Berl. klin. Wchnschr., 1886, **23**, 713.
[29] Deutsch. med. Wchnschr., 1891, **17**, 282.
[30] Münch. med. Wchnschr., 1909, **56**, 1955.
[31] Deutsch. med. Wchnschr., 1895, **21**, 244.
[32] Ztschr. f. Hyg., 1908, **61**, 219.
[33] Compt. rend. Soc. de biol., 1903, **55**, 35; 237; 259.

glass. The sputum and half of the water are then poured into a porcelain dish and as many drops *of 0.2 per cent sodium hydroxide* are added as there are cubic centimeters of sputum. For example:

	cc.
Sputum	10
Water	100
0.2 per cent sodium hydroxide	10 drops

The porcelain dish is then gently heated over a Bunsen flame with constant stirring. Little by little the remainder of the 100 cc. of water is added. The whole process of heating requires about 10 minutes.

The liquid is allowed to cool and its specific gravity is determined. If the gravity exceeds 1004 (that of the bacillus varying from 1010 to 1080), a little *50 per cent alcohol* is added until the density falls to 999 or 1000.

Enough liquid is now taken to fill two to four tubes which are centrifugated for 45 minutes to one hour. The sediment is spread upon slides and is dried and stained by the method of Ziehl-Neelsen.

Sputum is much more easily dissolved by the *antiformin* method of *Uhlenhuth and Xylander,*[34] (*mixture of calcium hypochlorite and sodium hydroxide*)[35] or by the still older method (1900) of Lannoise and Girard[36] with *Javel water*[37] one-third strength or pure, hot or cold, and with sodium hydroxide. The technique which I have adopted is the following:

Several cubic centimeters of sputum are mixed in a centrifuge tube with an equal quantity of a *30 per cent solution of antiformin* in water, or with *5 to 10 volumes of Javel water,* then shaken vigorously for two to three minutes and left in the incubator at 37°C. over night or for a few hours. The mixture then centrifugated. The supernatant liquid is poured off and replaced by a like quantity of physiological salt solution. The mixture is again shaken and centrifugated.

[34] Arb. a. d. k. Gsndhtsamte, 1909, **32**, 158.

[35] Antiformin is a disinfectant introduced in 1900 by Victor Tornell and Axel Sjoo, of Stockholm, for sterilizing brewery utensils. It oxidizes strongly. Javel water or pure Labarraque's fluid may be submitted for antiformin, but their action is less rapid.

[36] Presse méd., 1902, May 5.

[37] Also called Javelle's water. A solution of potassium hypochlorite (trans.).

The tubercle bacilli collect in the sediment, which is spread upon slides and fixed with heat. The antiformin should be diluted with distilled water in order that no acid-fast bacteria be introduced from outside.

After dissolving the sputum in antiformin, Loeffler prefers to shake the mixture with a little *chloroform* and *alcohol* (one part of chloroform and 9 of absolute alcohol) before centrifugating.

Bernhardt,[38] Haserodt,[39] Kawai,[40] Kinyoun, Jane L. Berry and Mary A. Smeaton[41] use a mineral oil, *ligroin* instead of chloroform. This enables one to dispense with centrifugation. With chloroform the bacilli are carried to the bottom. With ligroin, on the contrary, they are creamed and are found in the zone of separation of the two liquids, *underneath the ligroin which floats above.*

Many modifications of these procedures have been proposed. That of Lorentz gives good results.

To *5 cc. of sputum* add 15 cc. of *50 per cent antiformin;* shake vigorously until completely fluidified; heat until steam is emitted; centrifuge 10 minutes and spread the sediment upon slides. To do away with the air bubbles after fluidifying the sputum, it is recommended to add *15 cc. of alcohol* and then centrifuge.

The antiformin method is very practical and trustworthy and can be applied not only to sputum but also to pus from cold abscesses, to fragments of ground up body tissue and to feces. In searching for tubercle bacilli in the latter, one should take about 5 grams of material and dilute it first with 10 cc. of water; a considerable quantity of antiformin (about 30 cc. of a 40 per cent solution) is then added. After shaking, the mixture is allowed to digest for a couple of hours at 37°C., and is then centrifugated.

When tubercle bacilli are sought in *exudates* (pleural, spinal or joint fluid) antiformin should not be used, since the cellular elements must be preserved intact for cell count and differentiation. These factors are very important for diagnosis and the centrifuge alone should here be employed.

To demonstrate the presence of tubercle bacilli in the circulating blood, the best method consists in drawing off 10 or 20 cc. of

[38] Deutsch. med. Wchnschr., 1909, **35**, 1428.
[39] Hyg. Rundschau, 1909, **19**, 699.
[40] Med. klin. 1911, **7**, 142; 186.
[41] J. Infect. Dis., 1914, **14**, 159.

blood from an elbow vein, with a syringe, and adding it immediately to a tube containing 10 or 12 cc. of 2 per cent sodium citrate in physiological salt solution. The tube is stoppered with a sterile rubber cork; it is turned upside down two or three times and put in an ice box for 24 hours. The supernatant fluid is next carefully poured off and the sediment drawn up with a pipette, either for stained preparations or for inoculating animals.

Léon Bernard, R. Debré and Baron[42] prefer to treat the blood, immediately on its being drawn, with *20 cc. of 30 per cent alcohol* (to 10 cc. of blood). The laking of the cells is then completed by the gradual addition of *30 cc. of 40 per cent alcohol.* The mixture is next shaken vigorously and centrifugated for a half hour. After the supernatant fluid is poured off, the sediment is taken up in 40 cc. of 40 per cent alcohol; the mixture is shaken and one or two drops of a *1 in 10 alcoholic sodium hydroxide solution* are added. The centrifugation is repeated. The very small sediment thus obtained is spread upon a slide and stained.

When blood clots are to be examined for bacilli the method of *digestion in fluorated medium,* as proposed by Jousset,[43] may be employed. This method serves to digest the fibrin by means of a fluorated artificial gastric juice composed of:

Crystallized pepsin (Titre 50 of the French Pharmacopoeia)	1 to 2 gms.
Pure glycerine..	10 gms.
Hydrochloric acid	
Sodium fluoride	3 gms.
Distilled water	1 liter

Mix 10 to 20 volumes of this fluid with one volume of clot· leave in the incubator at 37° for three hours; centrifuge.

D. THE STAINING OF SECTIONS

If one wishes to study the relation of tubercle bacilli to the cellular elements in tissue, the latter should be cut into small cubes 0.5 cm. in thickness and fixed first in *60 per cent alcohol.* They are then passed successively, in the course of 24 hours, through *a series of 70 per cent, 80 per cent, 90 per cent and absolute alcohol.* The pieces may also be fixed from the start by being put in a *10 per cent solution*

[42] Bull. Soc. d'études scient. sur la tuberc., Nov. 1912.
[43] Semaine Méd., 1903, **23**, 22.

of formol for 24 hours and then in absolute alcohol. They are afterwards embedded in paraffin according to the usual histological technique (*alcohol-xylol, pure xylol, xylol-paraffin, paraffin*).

The sections should be as thin as possible and should be fixed on the slides by means of a little thymolated albumen. They are dipped in ether to dissolve out the paraffin, then in absolute, 80 per cent, 60 per cent and 40 per cent alcohol successively, and in distilled water. They are finally immersed in Ziehl solution where they should remain at least one hour at 37°C., or 24 hours at laboratory temperature. After being decolorized with an alcohol-hydrochloric acid solution (1 cc. of HCl in 100 cc. of 70 per cent alcohol) or still better in chlorhydrate of aniline (in 2 per cent aqueous solution) they are washed in 95 per cent alcohol; in water, and counterstained with the *carbolated blue of Kühne* for one minute, they are again washed in water, dehydrated rapidly in absolute alcohol, cleared in oil of cloves and, after a final and generous bath in xylol, mounted in Canada balsam. The bacilli stand out clearly red against a blue background.

It may be desirable, for purposes of study, to do a differential stain of the cell nuclei. In such a case the section is first treated for two minutes with *Delafield's hematoxylin* diluted to one third strength; then washed carefully in water and stained with *Ziehl* as described above, one hour at 37°C., etc.

The method of Herman-Caan[44] is also to be highly recommended. The technique is as follows:

First stain the section with *chlorhydric carmine* (*Mayer's carmine*) for 10 minutes.

Differentiate in *1 per cent hydrochloric alcohol* (1 cc. CHl pure in 100 cc. of 70 per cent alcohol) until the nuclei are distinctly apparent; wash; stain about two hours in a *solution of crystal-violet in ammonium carbonate* (3 parts of a 1 per cent solution of ammonium carbonate in distilled water and one part of a 3 per cent solution of crystal-violet in 95 per cent alcohol); decolorize a few seconds with a *10 per cent nitric acid solution* and next treat with *95 per cent alcohol* until the carmine color reappears;

Dry; mount in balsam.

[44] Centralbl. f. Bakt., 1909, **49**, 637.

E. DIFFERENTIAL STAINING METHODS

One of the essential characteristics of the tubercle bacillus is its so-called acid-fastness, in other words the property of retaining the stain, once fixed, despite treatment with acids or other decolorizing agents. This property is, however, not restricted to the tubercle bacillus.

Benian[45] and Hope Sherman[46] conclude, from studies of both ground up and unbroken bacilli, that this quality is of a purely physical nature and exists only when the bodies of the bacilli are intact.

There is to day a whole series of bacteria which are known to be equally acid-fast. Such is the case with the *leprosy* bacillus, discovered by Hansen; the bacilli of the skin to which Lustgarten, in 1884, attributed the etiology of *syphilis;* the *smegma* bacillus of Alvarez and Tavel (1885); that found by Gottstein (1886) in *cerumen* from the ear; certain bacilli found fairly commonly in *butter* and *milk* (Koch, Petri, Korn, Binot, L. Rabinowitsch, Kayserling), and in *manure* (the *mist bacillus* of Moeller).[47]

Acid-fast bacilli have also been found in *soil* (Karlinski, Moeller), in *sewage* (Spina, Houston) and also on certain *plants* (*Bacilli of the graminaceae: grassbacillus, Timothy bacillus of* Moeller).

Bacteriologists have long discussed the question whether the various acid-fast bacilli, some pathogenic, others saprophytes,—the latter grown easily and rapidly on artificial media,—have any common source or origin. The problem is not solved, but the answer up to now seems to be in the negative for the following reasons:

In spite of numerous attempts on the part of the most skilful investigators, no one has succeeded in producing a tuberculous infection, inoculable in series from one animal to another, with any one of the above acid-fast bacteria.

These bacilli, when inoculated into *non-tuberculous* animals, do not render the latter specifically sensitive to subsequent inoculations with true tubercle bacilli, a sensitization which characterizes

[45] J. Path. and Bact. 1912, **17,** 199.

[46] J. Infect. Dis., 1913, **12,** 249.

[47] See the monograph of Potet on this subject; Thesis, Lyons 1902; also Weber, Arb. a. d. k. Gsndhtsamte, vol. IX; L. Rabinowitsch, Centralbl. f. Bakt., 1899, **25** 77; and further in this book, chapters XXVIII and XXIX.

what we shall later study under the name of the *phenomenon of Koch* (*Chapter XXXIX*).

They are *not agglutinated* by sera of tuberculous individuals nor can they serve as *antigens* against tuberculous antibodies.

Their secretory products have no such toxic effect on tuberculous animals as has tuberculin made from the tubercle bacillus of Koch.

Finally, *inoculation of these acid-fast bacilli into the healthy organism does not produce the slightest resistance to true tuberculous infection.*[48]

Meanwhile there is nothing to prove that bacilli in their natural cycle and by slow adaptation to their parasitic life in the animal or human body, cannot become capable of producing tubercles or of transforming themselves little by little into tubercle bacilli. But this is only an hypothesis with no underlying experimental facts.

Quite different in its importance is the question of knowing on what criteria should be based the differentiation of the authentic tubercle bacillus from the acid-fast bacilli which resemble it and which are encountered so frequently in pathological material, in the excretions or in the secretions (sputum, feces, urine), on mucous membranes or on healthy or diseased skin. Indeed the staining reactions do not seem to permit of this differentiation. Several of the bacilli are less resistant to decolorization with acids than is the true tubercle bacillus, but such variations in acid-fastness are too slight to permit of assurance.

I should add however that it is often possible to distinguish the acid-fast bacilli found so commonly on the *genital mucous membranes*, especially of women (according to Grunbaum, they are found in urine of women in 59 per cent of cases) and also those of the *normal skin*, from the tubercle bacillus, by utilizing the technique of Dahms. This consists in immersing the slides prepared from centrifuged urine sediment for example, in absolute alcohol for three hours before any fixation whatever. The slides are next placed in a bath of *3 per cent chromic acid* during 15 minutes and then stained with *Ziehl fuchsin.* After decolorization with nitric acid diluted to one-fourth strength or with 2 per cent chlorhydrate of anilin and alcohol, the slides are counterstained rather longer than usual (about five

[48] Statements contrary to this assertion, published by Bayon (Soc. of Tropical Medicine, 1912) and by Fritzsche (Dissertation, Zurich 1908) are absolutely incorrect, according to the results of numerous experimenters. I have been able to satisfy myself that they are erroneous.

minutes) with a concentrated alcoholic solution of methylene blue. The acid-fast *smegma* bacilli have a blue tint, while the tubercle bacilli are distinctly red. Dahms insists too on the fact that the smegma bacillus never shows the curved forms which are so frequently observed with the tubercle bacillus.

Antiformin is also excellent for differentiating tubercle bacilli from the numerous varieties of *para-tubercle* and *acid-fast bacilli*, which will be discussed later. The *bacilli of smegma* for example, those of *Moeller* and of *Tobler*, the *acid-fasts of fecal matter*, are completely dissolved (as Anna V. Spindler-Engelsen[49] of Zurich, has shown) in a half hour in 15 per cent solution, while *human and bovine tubercle bacilli, even after four days of soaking in a 50 per cent solution of antiformin, are still to be recognized morphologically.* The *slow-worm bacillus* is a little more resistant that other paratubercle bacilli. It is however dissolved in 24 hours in the 50 per cent solution.

But,—and this will be the lesson of this chapter,—in the present state of our knowledge, the surest means of making an exact diagnosis lies in *experimental inoculation.*

One should regard as tubercle bacilli only those which, when introduced into the body of a susceptible animal, such as the guinea pig, produce tuberculous lesions further inoculable in series, that is to say capable of consecutively infecting susceptible animals, of the same or different species (guinea-pig, monkey), when made to pass from one to another.

[49] Centralbl. f. Bakt., 1915, **76**, 356.

CHAPTER II

CULTIVATION AND ISOLATION OF THE TUBERCLE BACILLUS

The tubercle bacillus exists ordinarily as a parasite of the lymphatic cells. Its cultivation on artificial media is slow and difficult, especially in the first generations. It can however be grown without too much difficulty from tuberculous tissue in which it exists in pure state. But its isolation requires quite delicate methods if it is to be accomplished from sputum or from open lesions where other microorganisms—such as the usual bacteria of pus—are present in large number and grow much more easily on the same media.

Robert Koch,[1] after numerous failures, was the first to succeed in obtaining a pure culture by crushing newly softened tubercles and smearing them over beef or sheep serum coagulated and sterilized by several successive heatings at 68°C. The serum was contained in small crystal dishes with glass covers and, after inoculation, was kept in an incubator at 37°C. On examining with a magnifying glass, after ten to fifteen days, Koch perceived some tiny grayish scaly colonies on the surface of the translucent media. When these were transplanted to other similar dishes, they produced a new growth, and this time more quickly, of irregular clumps of elevated colonies, always dry and scaly. Microscopic examination of the latter, after staining, showed bacilli identical with those in the tubercles from which the cultures had been taken. Furthermore their inoculation into the guinea pig and rabbit reproduced exactly the same lesions as were obtained when the ground-up pulp of tuberculous organs was introduced into the body of these animals, according to Villemin's technique.

Robert Koch tried other culture media. He deposited upon the surface of sterile fluid serum some of these scaly flakes from an initial culture on coagulated serum and obtained a scanty growth in the form of a thin extremely fragile film. But he did not succeed in growing the bacillus on broth or on nutrient agar, and after having described all his efforts he declared "that it is not to be hoped that

[1] Mitt. a. d. k. Gsndhtsamte, 1884, **2**, 66.

the cultivation of the tubercle bacillus is to play any very important part in the study of the disease."

Shortly afterward, Nocard and Roux[2] published in the *Annals of the Pasteur Institute* an important article wherein was described an improvement of technique to which we are indebted for great advancement in the study of tubercle bacilli and their secretory products. These scientists brought out the fact that glycerin added in proper proportion (5 to 8 per cent) to broth, agar or serum, renders the media particularly favorable for the growth of the germ.

A. CULTIVATION OF THE TUBERCLE BACILLUS FROM PATHOLOGICAL MATERIAL FREE FROM OTHER BACTERIA

Despite improvements in technique, it is always difficult to obtain an initial culture of the bacilli, even though the inoculation be made with tuberculous tissue in which the bacilli are present in a pure state.

The most approved means is by killing a tuberculous animal (by chloroform for example). The autopsy is quickly done, with all usual precautions as to asepsis, in such a way as to expose first the spleen, and then the principal groups of lymph nodes, especially those of the mediastinum. The surface of the spleen or node is seared with a red hot spatula and, through the cauterized area, some small bits are excised with a sterile scalpel. The pieces are put into a sterile rather thick glass tube, plugged with cotton, and are then minced to as finely homogeneous a pulp as possible by means of a sterile glass mixing rod with smooth tip. This pulp is smeared with a platinum spatula over the surfaces of several culture tube slants of 4 per cent glycerinated beef serum coagulated by heating at 70°C. The tubes are sealed with sterile rubber caps and incubated at 38°C. in an inclined, almost horizontal, position. All of the tubes thus planted do not show growth. But if they are carefully examined after 8 to 12 days, it is found that, on the surface of the serum of certain tubes, there are some small gray slightly elevated masses which are the colonies of bacilli. As soon as they are quite apparent their nature and purity are verified under the microscope. They should then be immediately transplanted to fresh culture media, where they develop more vigorously. To this end,

[2] Ann. de l'Inst. Pasteur, 1887, 1, 19.

PLATE I

1, 2, 3, 4. Cultures upon 4 per cent glycerin potato
- 1. Human tubercle bacilli.
- 2. Bovine tubercle bacilli.
- 3. Bovine tubercle bacilli (biliated).
- 4. Avian tubercle bacilli.

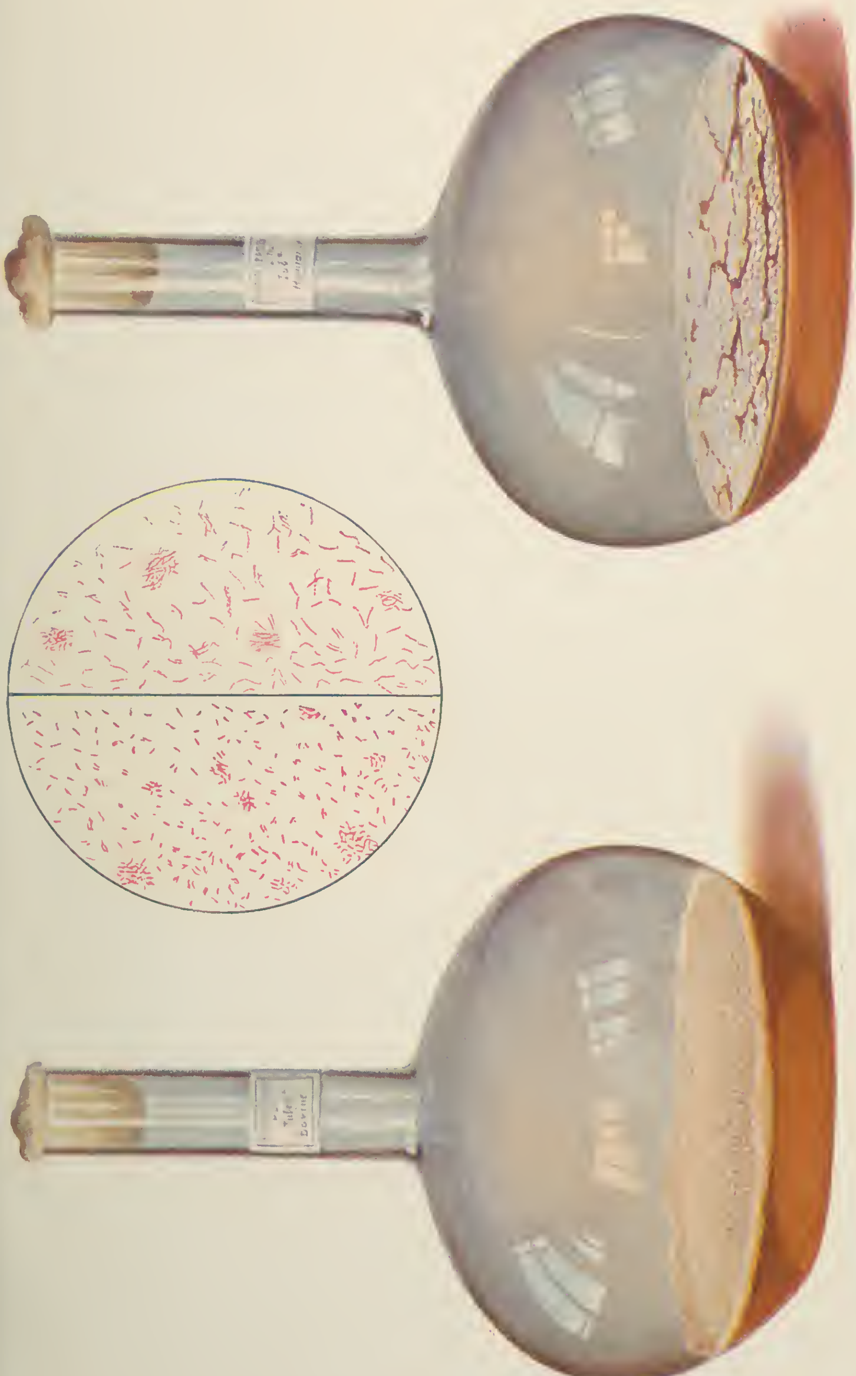
5, 6, 7. Cultures upon 4 per cent glycerin agar
- 5. Human tubercle bacilli.
- 6. Bovine tubercle bacilli.
- 7. Avian tubercle bacilli.

PLATE II

1. Culture of bovine tubercle bacilli upon glycerin broth. 1′ Preparation from same culture. Stained with Ziehl.

2. Culture of human tubercle bacilli upon glycerin broth. 2′ Preparation from same culture. Stained with Ziehl.


VII
VI
V
IV
III
II
I



IIa

Ia

II

I

a generous amount of the growth is taken on a flamed platinum spatula and smeared over the whole surface of a new tube of glycerinated coagulated serum which is in turn put in the incubator at 37°C. Usually, by the end of three or four weeks, the development of colonies is sufficiently abundant to permit of their inoculation into other tubes of serum, glycerin broth, or glycerin potato, and future cultures in series are readily obtained (*Plates I and II*).

The serial cultures, starting from an original good growth on coagulated glycerin serum, are best made on glycerin potato or glycerin broth.

The material which is to serve for the initial inoculations can be made richer in bacilli if, as Wedensky[3] showed, the piece of tissue (spleen or lymph node), aseptically excised, is suspended by a sterile wire in a large test tube or in an *Erlenmayer* flask containing a little glycerin broth, so that the tissue is only partly submerged. In one or two weeks the growth of bacilli is sufficient to permit of cultures being secured more easily.

a. Cultures on potato

The method of culture on glycerin potato was first described by A. D. Pawlowsky[4] who studied it in 1888 at the Pasteur Institute, utilizing test tubes constricted at the junction of the middle and lower thirds;—such tubes as Roux had already been using there since 1886.

Semi-cylindrical sections are cut out of large potatoes by means of a punch. After the skin is removed from the ends, each piece should be 5 to 6 cm. long and have a diameter corresponding to that of the interior of the tubes. The freshly cut sections should be quickly immersed in a dish containing a 1 per cent solution of sodium carbonate, where they are left to soak for one or two hours, then dried on a cloth and one piece put into each test tube. The latter should have been previously filled with broth, or 5 per cent glycerinated physiological salt solution, up to the constriction level. The tubes are plugged with cotton and sterilized once in the autoclave for 30 minutes at 120°C. after which they are sealed with sterilized rubber caps to prevent evaporation.

A rather stiff platinum spatula is used to inoculate the surface of the

[3] Centralbl. f. Bakt., 1913, **68**, 429.
[4] Ann. de l'Inst. Pasteur, 1888, **2**, 303.

potato. On this medium the tubercle bacillus grows more rapidly and much more abundantly than on coagulated serum, so that this medium is very useful for procuring large quantities of bacteria. In four to five weeks the surface of the potato is entirely covered with a thick coating of heaped up granular colonies which stand out irregularly and are of a grayish white color; at times they are dry or again moist, according to the origin of the bacilli, whether *human*, *bovine* or *avian*. On some varieties of potato, which contain a little glucose, the culture takes on a pink color approaching a brick red.

Glycerinated potato can serve for the initial cultivation of tubercle bacilli from the pus of cold abscesses, or from the ground up pulp of tuberculous organs free from other bacteria; but without any question the primary cultures are obtained with more certainty on glycerinated coagulated serum. In return, bacilli grown first on potato develop with much more vigor afterward when replanted upon the same media or upon glycerin broth.

b. Cultures on fluid media

It is only from cultures on fluid media that *tuberculin* can be prepared and the soluble excretory poisons of the bacillus studied. Consequently these media are much used in laboratories. The most widely employed is well cleared ordinary veal or beef peptone broth, to which 4 to 5 per cent of glycerin is added and which is rendered weakly alkaline, litmus serving as indicator.

Meat is not indispensable. Beck replaces it to advantage and very simply by 100 cc. of serum (from horse, ox or swine) which is added to 900 cc. of water and heated to boiling for one hour (in an unbolted autoclave). After filtration the following are added to the clear fluid:

	gms.
Citrate of magnesia	2.5
Asparagin	2.0
Glycerin	20.0

The mixture is again autoclaved for 15 to 20 minutes at 112°C. and filtered. The broth is portioned out in flat bottom flasks, each containing fluid to a depth of 2 cm. The flasks are plugged with cotton and sterilized for a half hour in the autoclave at 120°C. After cooling, each one is inoculated by carefully depositing, on the surface of the fluid, a few scaly fragments from a potato culture or a

fragment of the film from another culture on broth. This is done by means of a platinum loop or spatula. The thin films of recent growth or young cultures on solid media serve best for these plantings on broth. The essential point is not to submerge the piece transplanted since, if the latter is drowned in the mass of fluid, it will not grow.

The flasks are put in an incubator which can be kept at a constant temperature of 38°C. and which is rarely opened. After a few days a very delicate film can be seen to form about the transplanted fragment. At the end of 3 to 4 weeks the film covers the whole surface of the liquid and tends even to ascend on the sides of the flask; it then thickens itself by wrinkling more and more. In six weeks the growth is complete. There is no further increase. The film takes on the appearance of a layer of candle wax wrinkled like leather; it then becomes split up and at the slightest movement of the flask the fragments fall to the bottom. The subjacent fluid always remains perfectly clear. If it becomes cloudy, it is because the culture is contaminated by some other organism.

Repeated reinoculations on broth should always be made with very thin films of recent growth.

Attempts have been made to prepare fluid media whose chemical composition would be more constant than that of a meat infusion which always varies to a considerable degree. Kühne, Proskauer and Beck,[5] Uschinski, Hipp, Martin, C. Fraenkel, Von Schweinitz,[6] Löwenstein and Pick, have proposed complex synthetic formulae into which enter, along with various mineral salts, leucin, tryosin, asparagin, glycocoll, sarcosin, hippuric acid, certain sugars (glucose, galactose, maltose) and polyatomic alcohols (dulcite and mannite).

The preparation of Kühne's[7] media is begun by mixing the following:

	gms.
Sodium chloride	16.0
Magnesium sulphate, cryst	3.5
Calcined calcium sulphate	1.5
Calcined magnesia	2.5
Anhydrous potassium	62.13
Sodium hydroxide	7.35

[5] Ztschr. f. Hyg. 1894, **18**, 128.

[6] Centralbl. f. Bakt., 1893, **14**, 330.

[7] Ztschr. f. Biol., 1892, **30**, 221.

	gms.
Reduced iron	6.2
Phosphoric acid (specific gravity 1.3)	95.0
Lactic acid (specific gravity 1.2)	50.0 to 60.0
Distilled water	600.0

Heat to boiling.

Twelve cubic centimeters of this mixture represent about 10 grams of extract of meat, that is to say the quantity necessary to prepare one liter of nutrient solution.

To the 12 cc. are added (for one liter).	gms.
Leucin	4.0
Tyrosin	1.0
Asparagin	2.0
Ammonium succinate	2.0
Taurin	0.5
Glycerin	40.0
Sodium chloride	5.0

The mixture is rendered weakly alkaline with sodium hydrate, is sterilized and distributed into flasks.

The medium of Proskauer and Beck contains the following elements:

	gms.
Ammonium carbonate	0.35
Magnesium sulphate	0.25
Potassium phosphate, (mono)	0.15
Glycerin	1.50
Water	100.0

For the preparation of albumin-free tuberculin (tuberculin A. F., *albumosefrei*), which will be discussed later (*Chapter V*), this medium was modified in Koch's laboratory as follows:

	gms.
Potassium phosphate, (mono)	0.50
Magnesium sulphate	0.06
Magnesium citrate	0.25
Asparagin	0.50
Glycerin	2.0
Caustic soda	0.25
Distilled water	100.0

The medium which I have studied with L. Massol and M. Breton is very satisfactory and is easily prepared.[8]

[8] Compt. rend. Soc. de biol., 1909, **67**, 580.

Its composition, per liter, is as follows:

	gms.
Sodium carbonate	1.0
Ferrous sulphate	0.040
Magnesium sulphate	0.050
Potassium phosphate	1.00
Sodium chloride	8.5
Glucose	10.00
Glycerin	40.00
Witte's peptone	10.00
Distilled water	1000.0

The peptone may be replaced by 2 gms. per 1000 of succinimid or by 2 gms. per 1000 of asparagin, but with the latter the sodium carbonate must be left out.

On this medium growth is very abundant.

L. Massol and M. Breton[9] have shown that glucose and levulose may be used to replace glycerin for cultures on potato. But this does not apply to saccharose. However if one takes care to invert the latter, culture is then possible and the growth is as abundant as in the presence of glucose. The tubercle bacillus therefore does not produce any *invertin.*

Potassium and *magnesium* appear to be the mineral elements most necessary for the growth of the bacillus on artificial media, since there is no growth when they are lacking (Bezançon, Philibert and Boudin).[10]

Baudran[11] has prepared another medium on a basis of glycerophosphates, taking advantage of the fact that the bacillus utilizes phosphorus-containing organic bodies in its growth. In glycerin cultures, the glycerin is oxidized and converted into glycero-phosphoric acid, and the latter is the source of the lecithin which is a constituent of the body of the bacillus.

The medium of Baudran has the following composition:

	gms.
Glycero-phosphate of sodium	2.24
Glycero-phosphate of calcium	1.20
Glycero-phosphate of potassium	0.60
Glycero-phosphate of magnesium	1.76

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[9] Compt. rend. Soc. de biol., 1911, **71**, 340.

[10] Bull. Soc. d'étude scient. de la tuberc., 1913, Feb. 13.

[11] Compt. rend. Acad. des sci., 1910, **150**, 1200.

	gms.
Byla's albumose	10.00
Glycerin	50.00
Sodium citrate	4.00
Water	1000.0

The glycerin may be omitted if the quantity of sodium glycerophosphate is increased to 10 gms. and that of the sodium citrate to 8 gms.

Tiffeneau and Marie[12] defined the conditions of bacillary growth in a mineral medium similar to that of Proskauer and Beck and of which the formula is as follows:

	gms.
Monopotassium phosphate	5.0
Citrate (or sulphate) of magnesium	2.5
Mannite	6.0
Ammonium sulphate	2.0
Glycerin	15.0
Water q. s. ad	1000.0

These workers found that, while cultures in peptone broth require definite alkalinity, the opposite is true with regard to the above mineral glycerin medium. The optimal acidity titrated with sodium hydroxide solution (phenolphthalein as indicator) varies between 0.05 to 0.08 per 100.

A. Frouin[13] obtains growth on a medium based on a combination of glucosamin and sarcosin. This medium has the following composition:

	gms.
Sodium chloride	6.0
Potassium chloride	0.30
Disodium phosphate	0.50
Magnesium sulphate	0.30
Calcium chloride	0.15
Glycerin	40.00
Glucosamin	2.00
Sarcosin	2.00
Water	1000.0

This solution, after being neutralized, sterilized, filtered, portioned out in flasks and sterilized anew, is perfectly suited to the growth of the tubercle bacillus.

[12] Compt. rend. Soc. de biol., 1912, **72**, 48.
[13] Compt. rend. Soc. de biol., 1910, **68**, 915.

P. Armand-Delille, A. Mayer, G. Schaeffer and E. Terroine[14] have tried to determine what elements entering into the composition of peptone broth are indispensable to the growth of the germ. From their experimental results, they conclude that the purin bases appear to be of no importance, while the diamino acids (arginin, histidin) have a markedly favorable effect on the cultures, and the true extractive substances are of capital importance. Among the latter the most essential are creatin, carnosin and sarcosin. The first of these has the effect of rendering the growth more abundant, while sarcosin hastens it. Inosite and glucose, the latter especially, appear also to have a distinctly favorable influence. The medium which in the minds of these investigators combines the greatest number of advantages, has the following composition:

	gms.
Water	250.00
Sodium chloride	1.25
Magnesium citrate	0.60
Monopotassium phosphate	1.25
Glycocoll	0.50
Aspartic acid	0.50
Nitrate of carnosin	0.10
Creatin	0.10
Sarcosin	0.10
Glucose	0.50
Inosite	0.10
Glycerin	10.0
1 per cent sodium hydroxide solution	1.0 cc.

In a more recent publication,[15] the same authors state that they have obtained growths still more quickly and more abundantly on the following medium which contains a mono-amino acid (glycocoll) and a diamino acid (arginin):

	gms.
Water	250.00
Sodium chloride	1.25
Monopotassium phosphate	1.25
Magnesium citrate	0.60
Glucose	1.00
Glycerin	10.00

[14] Compt. rend. Acad. des sci., 1912, **154**, 537.
[15] Compt. rend. Soc. de biol., 1913, **74**, 272.

	gms.
Glycocoll	1.00
Arginin	0.50
1 per cent solution of sodium hydroxide ... 1 cc. (added after previous neutralization)	

In twelve days, on this medium, the bacilli form a complete thick wrinkled pellicle reaching up on the sides of the flask. Virulence is well preserved and the fluid on evaporation furnishes an active tuberculin.

B. Sauton[16] showed, as Léon Massol and I had already discovered, that it is desirable to add a small quantity of iron to the mineral media in order to obtain abundant growth. The one which he composed contains, per liter:

	gms.
Asparagin	4.0
Glycerin	60.0
Citric acid	2.0
Dipotassium phosphate	0.5
Magnesium sulphate	0.5
Iron citrate (ammoniacal)	0.05

After 20 days of growth, the weight of the bacilli obtained on this medium is about 1 gm. (in the dry state) per 100 cc. of the liquid, while on glycerin broth the weight is only 0.65 gm., and on the fluid of Proskauer and Beck only 0.35 gm. According to B. Sauton the presence of iron in a proportion of 1 to 100,000 is sufficient to triple the weight of the growth.

Malm (of Christiania)[17] finds that tubercle bacilli refuse to grow in media devoid of phosphorus and that the presence of a small quantity of silicate of potassium exerts a very favorable influence. In fluid media deprived of albumin, the bacillus forms it, and the tuberculin obtained can be precipitated with alcohol in the form of a white powder, toxic for tuberculous animals. The alcoholic filtrate contains no albumin and is non-toxic. *From this it is evident that the tuberculin is contained in the substance of albuminoid nature formed by the bacillus.* Malm considers tuberculin as chiefly an exchange product of the tubercle bacillus and not an extract of its protoplasm.

With albumin-free artificial media which contain asparagin, glycerin, and 1 per cent of mannite or dextrose, for example, Kendall

[16] Compt. rend. Acad. des sci., 1912, **155**, 1860.

[17] Centralbl. f. Bakt., 1913, **70**, 141.

Day and Walker[18] have shown that the tubercle bacillus forms the nitrogenous elements which enter into its composition at the expense of the asparagin, and the fats and waxes at the expense of the glycerin and sugars. The bacillus secretes a *lipase* which splits ethyl butyrate and, although weakly, castor oil. This lipase is also found in cultures of other acid-fast bacilli, such as bovine and avian tubercle bacilli, the paratubercle bacilli of the skin, smegma, grass bacilli, etc. Its presence can even be demonstrated in culture filtrates. It is thermostabile and increases in amount in proportion to culture development; but after having reached a maximum, it decreases in amount and then disappears. It is not present in old cultures.

If one does not propose to study the products of secretion of the bacillus and is only trying to cultivate it from tuberculous organs, it is always preferable to employ coagulated glycerinated serum, or glycerin potato. The egg medium of Dorset,[19] the preparation of which is to be described, can be used very conveniently in certain cases.

c. *Cultures on egg media*

Hens eggs are carefully scrubbed with a brush in boiled water and then immersed for some minutes in a 5 per cent carbolic acid solution. They are next taken between two sheets of sterile blotting paper, the two ends flamed and a hole made in each with pointed flamed forceps. By means of a rubber tube containing sterile cotton as a filter, the contents of the egg are blown into a previously weighed sterile *Erlenmayer* flask. The egg should be blown from the upper or air chamber end. A quantity of water equal to 10 per cent of the weight of the egg is next added to the flask, which is shaken to assure a homogeneous mixture of the yolk and white and the water, care being taken to avoid air bubbles. The whole is then put through heavy sterile muslin in a funnel and the filtered mass portioned out aseptically in test tubes. The tubes are coagulated by being put in a thermostat, in an inclined position, at 70°C. for two hours. They are then left in the incubator at 37°C. for three days, in order to detect any contamination. Finally they are sealed with rubber caps and kept in a vertical position until used.

[18] J. Infect. Dis., 1914, **15**, 417.
[19] Amer. Med., 1902, **3**, 555.

Lubenau[20] also highly recommends this culture medium but modifies its preparation in that for each egg, he adds 30 per cent of its weight of alkaline broth glycerinated to 5 per cent.

The use of the egg for cultivating tubercle bacilli had already been proposed in 1896 by Capaldi. Bezançon and Griffon[21] utilize only the yolk which they mix raw with glycerin agar in the proportion of one part of yolk to two parts of agar. On this medium the human bacillus grows rapidly, the colonies being moist and greasy, instead of dry and scaly as on glycerin serum or ordinary glycerin agar.

Besredka,[22] with the collaboration of F. Jupille, made a very interesting study of a mixed medium composed of 100 gms. of meat broth without peptone, to which are added *cold* a mixture of 20 cc. of a 10 per cent emulsion of egg white and 5 to 20 cc. of another 10 per cent emulsion of egg yolk, each mixture having been separately filtered and sterilized at 115°C. for 20 minutes.

Upon this liquid medium, which is devoid of glycerin and salt, and which can be hardened, if desirable, by adding 1 per cent of agar, growth is from the outset obtained *deep in the fluid* and as abundantly as that of the streptococcus in broth. In 2 or 3 weeks, the cultures form a whitish membrane which completely covers the bottom of the flask and can be converted into a fine powder with a little shaking. In this fluid bovine bacilli assume a peculiar appearance of glairy threads, adhering to the sides of the flask, and of a muco-membranous consistency. Human bacilli, on the other hand, after 4 to 6 weeks, form small scales which do not adhere to the glass.

The characteristic odor developed by tubercle bacilli in ordinary media is totally lacking with this medium of Besredka. And yet a very active tuberculin is elaborated, since after incubation at 38°C. for 3 to 4 weeks, 1.5 to 2 cc. of the filtered non-concentrated fluid suffices to kill tuberculous guinea pigs in less than 24 hours after intraperitoneal injection.

We shall see later that the egg media are of special use when it is necessary to determine whether tubercle bacilli are of human or bovine origin.

[20] Hyg. Rundschau, 1907, **17**, 1456.

[21] Compt. rend. Soc. de biol., 1903, **55**, 603.

[22] Compt. rend. Acad. des sci., 1913, **156**, 1633; Ann. de l'Inst. Pasteur, 1913, **27**, 1009; 1914, **28**, 576.

d. Cultures on tissue

It was natural to attempt to grow bacilli on animal tissues. A. and L. Lumière[23] succeeded with pieces of liver and spleen from ox or calf. The tissues were cooked for 40 minutes in an autoclave, then cut up into square prisms, washed in distilled water, immersed for one hour in 6 per cent glycerin water and finally sterilized in potato tubes for 15 minutes at 120°C. On such a medium growth is very rapid.

P. Gioelli (of Genoa)[24] prefers pieces of human placenta in 6 per cent glycerin broth. Growth is observed after the fourth day, no matter whether the bacilli are from cultures or from tissue pulp.

e. Cultures in collodion bags and in filter bougies

I have finally to call attention to the procedure of growing the bacillus *in vivo*, as employed by G. Moussu[25] (of Alfort). This consists in inoculating glycerin broth in very thin sterile collodion bags which are introduced aseptically into the peritoneal cavity of the guinea pig, rabbit, sheep or ox. Instead of collodion bags, small porcelain filter bougies (*Chamberland* filter L2) may be used. After being filled and inoculated they are sealed with rubber and Golaz wax. The bags or the candles may be left for several weeks in the peritoneal cavity without harm to the animal. While the bacilli are developing, their dialysable secretory products are diffused out and soluble substances are taken in from the body fluids.

f. Cultures on various organic media—bile media

Other nutritive substances of animal or vegetable nature, whether solid or liquid, can be used for the culture of the tubercle bacillus, for example *milk*, its fats for the most part centrifuged off, glycerinated up to 2 or 3 per cent and sterilized; or again glycerin agar or glycerinated turnip or carrot. But these different media are of no special interest.

It is quite otherwise as regards a method which I have studied with C. Guérin[26] and which possesses the double advantage of enabling

[23] Comp. rend. Soc. de biol., 1906, **60**, 568.
[24] Policlinico, 1907, **14**, sez. med., 118.
[25] Compt. rend. Soc. de biol., 1906, **61**, 96.
[26] Compt. rend. Acad. des sci., 1908, **147**, 1456.

one to determine at the start whether the bacillus is of human or bovine origin and to progressively modify its virulence as desired. The method consists in inoculating the tuberculous material upon potatoes cooked in 5 per cent glycerin bile and leaving the culture to grow in the presence of an excess of biliary fluid. According as one uses human or bovine bile, only the human or bovine bacillus can be grown on the media. Our technique is as follows:

The contents of several gall bladders, as fresh as possible,[27] are put into a large round flask which is sterilized at 120°C. and put aside undisturbed for 3 weeks at laboratory temperature. An abundant sediment of brick red pigments is formed and this should be filtered out with paper just before use.

Pieces of potato, cut out with a punch, are completely immersed in this bile to which 5 per cent of glycerin is added. The whole is left in a water bath at 75°C. for 3 hours. The potatoes are next allowed to drain and are portioned out among the special potato tubes, which are filled up to the line of constriction with bile glycerinated to 5 per cent. The tubes are finally sterilized for 30 minutes at 120°C.

When sown upon potatoes prepared in this way, tubercle bacilli increase very rapidly and the growth takes on an altogether peculiar appearance which is in no way like that presented by ordinary cultures on glycerin potato. By the end of 10 days the whole surface is covered with a thin creamy greenish-gray layer of growth which thickens little by little to reach its maximum at the end of 45 days. At that time the potato is covered with a uniform glossy coating, of a light buff color and resembling an old culture of *glanders* bacilli.

[27] According to Daniel Brunet and C. Rolland (Compt. rend. Acad. des sci., 1911, **153**, 900) bovine bile has the following composition:

		gms. per 1000
Ash		12.5 to 14.3
Chlorides (as NaCl)		2.38 to 2.68
Phosphates (as P_2O_5)		1.31 to 1.58
Total nitrogen		2.3 to 2.5
Iron		0.016 to 0.018
Fat residue		27.80 to 28.80
Bile salts (tauro- and glycocholate of sodium)		15.30 to 15.80
Biliary nucleo-proteid		1.15 to 2.25
Lipoids		1.100 to 2.130
Of which	Cholesterins	0.410 to 0.813
	Lecithins and neutral soaps	0.690 to 1.317

The quantity of bacteria obtained from a single tube of potato medium is about 0.5 gm. (bacteria weighed in moist state).

Bacilli so cultivated are granular, slender and somewhat longer than those grown on the usual media. They preserve the same staining characteristics with Ziehl. Weight for weight, they give a higher proportion of fatty matter soluble in alcohol. Transplanted back upon ordinary media, they quickly reassume the appearance which they normally have on each of them. On glycerin broth, however, a first inoculation below the surface yields small grumous masses not unlike cultures of actinomyces. The second transplant to broth produces a film.

Such are the media most serviceable for the culture and study of tubercle bacilli, whether the latter be derived from other cultures, from body tissues or from material in which the bacilli exist in pure state.

B. CULTURE OF THE BACILLUS FROM PATHOLOGICAL MATERIAL WHICH CONTAINS OTHER BACTERIA

If the tubercle bacillus is to be isolated from material in which there are other pathogenic or saprophytic microörganisms, recourse must be had to methods which permit of eliminating the latter without harming the vitality of the bacillus of *Koch*.

The material may be sputum, pus from a lung cavity or from any abscess communicating with the exterior, fecal matter or urine. The procedure most to be recommended is that with antiformin, as proposed by Uhlenhuth.[28]

A measured quantity of sputum, 5 to 10 gms., is mixed in a sterile centrifuge tube with an equal quantity of a 30 per cent solution of antiformin. The tube is corked with rubber, shaken vigorously and allowed to stand for one hour; then, after centrifugation, the supernatant fluid is poured off and the sediment washed twice with sterile water. After the final centrifugation, the sediment is spread with a spatula on tubes of glycerin coagulated serum or on a 5 per cent glycerin potato medium. The antiformin destroys almost all the contaminating bacteria and one usually succeeds in this way in obtaining pure cultures from the start. Just as soon as the colonies are visible they should be inoculated upon new media.

Weber and Dieterlen[29] recommend the still simpler procedure of

[28] Arb. a. d. k. Gsndhtsamte, 1909, **32**, 158.
[29] Tub. Arb. a. d. k. Gsndhtsamte, 1912, H. 12, 1.

treating the sputum with 4 per cent antiformin. After one hour of contact the mixture is centrifugated and the sediment inoculated.

Dongès[30] has correctly called attention to the fact that certain strains of tubercle bacilli, both bovine and human, are more resistant to antiformin than are others. He was able to study several in which vitality was destroyed only after 12 or even 24 hours in a 15 per cent solution.

The medium of Hesse[31] can also be utilized. It is composed of:

Heyden nutrose	5 gms.
Normal solution of sodium hydroxide (crystallized at 28.6 per hundred)	5 cc.
Sodium chloride	5 gms.
Glycerin	30 gms.
Agar	10 gms.
Distilled water	1000 cc.

On this medium, poured into Petri dishes, a pure growth is frequently visible within 4 to 6 days, provided the sputum is inoculated immediately after being freshly collected in a sterile container. The mouth of the patient should be washed beforehand with dilute hydrogen peroxide.

The medium proposed by S. A. Petroff,[32] at the 1915 meeting of the Society of the American Bacteriologists, is likewise to be recommended. According to Petroff, it enables one to quickly isolate tubercle bacilli from sputum by virtue of the inhibitive action of gentian violet on the other microörganisms.

An infusion is first prepared from 500 gms. of beef or veal in 500 cc. of water containing 15 per cent of glycerin. The meat is left to macerate for 24 hours; it is then put through a sterilized press and the fluid collected in a sterile vessel.

Meanwhile the shells of several clean fresh eggs are sterilized by immersing them for 10 minutes in 70°C. alcohol, and the eggs are broken into a sterile vessel. The whites and the yolks, mixed by shaking, are filtered through sterile gauze on a funnel, and the egg mixture added part for part to the meat infusion.

To this last combination a 1 per cent alcoholic solution of gentian violet is added in the proportion of 1 to 10,000.

The medium is tubed and kept in an inclined position in an oven

[30] Ztschr. f. Hyg., 1913, **75**, 185.
[31] Ztschr. f. Hyg.., 1899, **31**, 502.
[32] J. Exper. Med., 1915, **21**, 38.

at 85°C. until completely hardened. On each of the two succeeding days it is kept at 75°C. for one hour.

To grow the bacillus from sputum, the latter (as fresh as possible) is diluted with an equal quantity of a 3 per cent solution of sodium hydroxide, left for a half hour in the incubator at 37°, neutralized to litmus with hydrochloric acid, centrifugated and the sediment divided among the culture tubes with a drawn out pipette.

C. Spengler (of Davos)[33] published in 1903 an ingenious procedure based on the fact that formaldehyde exercises its antiseptic action on saprophytic bacteria much more rapidly than on tubercle bacilli. His method is as follows:

In the bottom of a *Petri* dish, completely covered beforehand with a layer of filter paper, about 3 cubic centimeters of sputum are spread to a depth of two and a half millmieters. Pancreatin is sprinkled on the sputum to hasten digestion of the mucus. The inside of the cover of the dish is also fitted with a layer of filter paper which, after the action of the pancreatin (5 or 6 hours in the incubator at 37°C.), is moistened with 3 to 5 drops of commercial formalin. The dish is then kept at a temperature of 20 to 25°C. for two hours. This suffices to kill all the bacteria, except the tubercle bacilli, which can be directly planted upon coagulated glycerin serum in order to obtain pure cultures.

S. Piatkowski,[34] inspired by this method, proceeds in the following manner:

He emulsifies the bacterial mixture to be studied in 10 cc. of water or broth; to this he adds 2 or 3 drops of formalin and shakes vigorously. Half an hour later, the fluid (or better, one part of the sediment after centrifugation) is inoculated upon ordinary agar while another part is inoculated upon glycerin agar. The tube is shaken anew and the sowings repeated every quarter of an hour. Thus he obtains a series of tubes of which some may give a pure culture of acid-fast bacilli, all the other bacteria having been killed by the formol. Many acid-fast bacilli which do not produce tuberculosis grow rapidly on ordinary agar. They are thus readily differentiated.

C. Spengler showed that sputa can be incompletely sterilized by heat in a manner to kill all bacteria except the tubercle bacillus. An amount of nummular sputum the size of a hazel nut is taken

[33] Ztschr. f. Hyg., 1903, **42**, 90.

[34] Deutsch. med. Wchnschr., 1904, **30**, 878.

up on a platinum loop and held to the flame in such a way that it is roasted over its whole surface without being detached from the loop. This procedure is repeated two or three times and the desired sterilization is obtained.

The flamed mass is then put upon glycerinated serum and crushed. This method is possible but it requires practice. The antiformin method is certainly much more simple and more practical.

I have still to mention the method studied by F. W. Twort who isolates the tubercle bacillus directly by means of *ericoline,* a glucoside which kills the associated bacteria. A fragment of sputum is put into a 2 per cent aqueous solution of this substance, kept at 38°C. for one hour and inoculated directly upon Dorset's egg medium.

There is also the method of F. Ditthorn and W. Schultz[35] which consists in diluting the sputum with an equal quantity of water, adding a 15 per cent caustic potash solution up to 10 per cent of the volume of the sputum water mixture, then heating for 10 to 20 minutes in a water bath at 47 to 50° in order to render the whole homogeneous. To 30 cc. of this emulsion are added 1.5 to 2 cc. of a 2 per cent solution of iron oxychloride. A precipitate is formed which is collected and spread on slides without the necessity of centrifugating.

All of these methods, except that of Hesse and that of C. Spengler, are applicable to the isolation of tubercle bacilli from fecal matter, from urine, from the pus of lung cavities, from pneumothorax or from abscesses. For fecal matter the antiformin method is the most suitable. One should take about 10 gms. of material which is diluted in 20 cc. of sterile water and left in 30 cc. of a 20 per cent antiformin solution for about one hour. After being shaken vigorously, the mixture is centrifugated with aseptic precautions. The sediment is washed in sterile physiological salt solution, centrifugated a second time and (after pouring off the supernatant fluid) inoculated upon the different solid culture media (coagulated serum, potato, Dorset's egg, Lubenau's egg and potato with bile).

For urine, a sufficiently large quantity (about 100 cc.) must be centrifugated to begin with. The sediment is taken up in several cubic centimeters of water to which is added an equal quantity of 20 per cent antiformin. The further procedure should be as already described.

[35] Centralbl. f. Bakt. 1917, **79**, 166.

CHAPTER III

INFLUENCE OF PHYSICAL AND CHEMICAL AGENTS UPON THE TUBERCLE BACILLUS

A. ACTION OF AIR AND OF ATMOSPHERIC PRESSURE

The tubercle bacillus is very largely aerobic and can be grown only in media and in containers which permit of plenty of air. Furthermore, within the body it is in the very vascular organs, to which the blood stream brings a large supply of oxygen, that the bacillus develops most readily. However it is not a strict aerobe, since it vegetates under relatively anaerobic conditions, for example in the tissues of certain viscera such as the spleen, the liver and the kidney. Hueppe,[1] too, was able to cultivate it by direct planting upon the whole egg, the minute inoculation hole in the shell being carefully sealed with wax.

By maintaining cultures during a series of successive generations at a temperature of 38°C. and under a pressure of two and one-half atmospheres, S. Arloing[2] demonstrated that the bacilli assume elongated and irregular forms, frequently taking on a clubbed or conical shape and resolving themselves after a while into granules or spherical bodies. If such modified forms are again placed under ordinary atmospheric pressure and on new media, they reproduce normal bacilli.

B. ACTION OF LIGHT AND ULTRA VIOLET RAYS

As early as 1890, in a communication to the International Congress at Berlin, Robert Koch called attention to the fact that tubercle bacilli die rather quickly when directly exposed to the sun's rays, and more slowly when exposed to diffuse light. Cultures are very sensitive to it. Two hours of summer sun are sufficient to render them sterile (I. Straus). Bacilli contained in sputum and spread thinly on glass slides are destroyed in 10 minutes under the same

[1] Internat. Congr. Hyg. & Dem., 9th, Lond., 1891.

[2] Compt. rend. Acad. des Sci., 1908, **146**, 100.

conditions. According to Migneco's[3] experiments, linen and woolen cloths, soiled with tuberculous matter, then dried and cut into small squares and introduced under the skin of guinea pigs, are no longer capable of infecting the animals after 24 to 30 hours of exposure to full daylight.

Ultra-violet rays are strongly bactericidal. According to M. and Mme. Victor Henri and Baroni,[4] the rays emitted by the mercury vapor quartz lamp cause the bacilli to lose their acid-fastness in a few minutes and suffice to kill them in ten minutes; although the Gram-positive granules of Much remain and can still be stained (Rochaix and Colin).[5]

C. ACTION OF LOW TEMPERATURES

A dry cold temperature, even liquid air itself, does not destroy either the vitality or the virulence of the tubercle bacillus. According to Galtier, Cadéac and Malet, and Moussu,[6] temperatures in the neighborhood of minus 180°C, (temperature of liquid air and of liquid nitrogen), prolonged from a few hours to 8 days, with alternate freezings and thawings as well, do not affect the vitality of bacilli contained in tuberculous lesions.

D. ACTION OF HEAT

The optimal temperature for cultivating the tubercle bacillus of mammals is 38°C. Above 42°C. and below 30°C. growth ceases. The avian bacillus on the other hand grows very actively between 40° and 42°C. Growth ceases at 45°C. The thermal requirements of these bacteria are therefore very rigorous. Consequently if one wishes abundant and uniform cultures, one must have a well regulated incubator the doors of which are opened as seldom as possible.

High temperatures have very different effects depending on whether the bacilli are exposed *in a dry or moist environment*, or in *cultures* or tuberculous *products*. In order to definitely determine their resistance in the dry state, Krumwiede[7] triturated cultures

[3] Riforma med., 1895, No. 169, 227.
[4] Compt. rend. Acad. des sci., 1910, **151**, 724.
[5] Ibid., 1911, **153**, 1253; 1530.
[6] Compt. rend. de la Caisse nat. d. recherches sci., 1912.
[7] J. Infect. Dis., 1911, **9**, 115.

from eight different sources, dried them in an incubator for 24 hours and then ground them anew. They were portioned out in narrow drawn out tubes, not sealed, but bent at one end. Each tube contained 25 mgms. of bacteria. The dried organisms were now heated at 100°C. on a water bath for varying periods, air being allowed to escape freely from the tubes. After cooling, suspensions in physiological salt solution were injected into guinea pigs. The animals inoculated with bacteria heated 20 minutes showed slightly active circumscribed lesions. Those inoculated with bacilli heated 45 minutes remained definitely free from infection.

Similar experiments, under a variety of conditions, had previously been performed by Schill and Fischer, Woelsch, Straus and Gamaléia, Yersin, J. Forster, Th. Smith, Grancher and Ledoux-Lebard, and others. The conclusion reached was that above 80°C. the vitality of the bacillus diminishes rapidly; the higher the temperature or the longer the exposure, the quicker the loss of vitality.

In a *moist environment*, a prolonged exposure of 12 hours at 50°C. or 4 hours at 55° C., one hour at 60° C., 15 minutes at 65°C., 10 minutes at 70°C., 5 minutes at 80°C. or one minute at 95°C. is sufficient to destroy without fail the vitality of the bacillus in culture suspensions. Heat acts less rapidly on tuberculous products because of the protection afforded by the albumin. At every temperature also, the duration of the heating is a factor of prime importance and the degree of heat must be maintained throughout the entire mass of fluid. As regards *milk*, Forster[8] insists quite rightly on the unreliability of the heating contrivances used and on the necessity of maintaining a temperature of 70°C. for at least 30 minutes, if one wishes to be sure of destroying the bacilli.

E. EFFECT OF DESICCATION

Tuberculous sputum smeared on glass slides or on cloth and then dried at low temperatures in a dim light, retains its virulence from two to four months. If exposed to diffuse light, as in an apartment for example, it remains virulent for about 39 days, according to Twitchell. Beyond 60 days it can no longer infect guinea pigs.

In the dry dust of offices or public places, streets, etc., in poor light, the bacilli survive scarcely longer than 10 days. This also

[8] Centralbl. f. Bakt., 1909, **51**, 417.

applies to bacilli deposited upon the pages of books or on clothes (F. Kirstein[9]).

P. Chaussé[10] smeared drops of tuberculous sputum over slides which he dried, some at room temperature, and others in an incubator at 37°C. He then again took the material up in suspension and tested its comparative virulence for guinea pigs, both by injection and inhalation. He found that the two test methods give very different results. When the tests are made by injection, the bacilli dried at 15 to 20°C. in diffused light may infect the animal after 30 to 40 days; those dried in the dark remain virulent up to 60 days; those dried in the incubator are no longer virulent after 15 days. When the test is carried out by inhalation it is found that sputa dried at ordinary temperature are non-infective after 10 days and that those dried in the incubator at 37°C. are harmless after 4 days. Chaussé concludes from his experiments that the vitality of tubercle bacilli in sputa persists long enough (seven days on the average) to make it necessary to destroy them. But since this persistence of vitality is not very protracted, it may be said that *disinfection of the home, as a prophylactic measure,—which involves certain difficulties of execution,—can be replaced to advantage by the necessary regulations regarding spitting.*

F. INFLUENCE OF PUTREFACTION

Galtier, Cadéac and Malet, and A. Gaertner performed numerous experiments with a view to determining the duration of the vitality of the bacilli in cadavers and in tuberculous organs in a state of putrefaction. After 167 days of burial under ground, virulence was not destroyed. Schottelius[11] states that the bacilli remain alive several years in tuberculous corpses.

The phenomena of disintegration of sewage, whether in septic tanks or spread as manure, destroy them only with extreme slowness. According to Muschold,[12] and also to my own experiments, bacilli may continue virulent in wet mud for more than 4 months, and in garden soil for more than 7 months. A. Davos, F. Jessen, and Lydia Rabinowitsch[13] were able to infect guinea pigs by injecting particles of slime collected 100 meters below sewer outlets.

[9] Ztschr. f. Hyg., 1905, **50**, 186.
[10] Compt. rend. Acad. des sci., 1912, **155**, 486.
[11] Deutsch. med. Wchnschr., 1890, **16**, 226.
[12] Arb. a. d. k. Gsndhtsamte, 1900, **17**, 56.
[13] Berl. klin. Wchnschr., 1910, **47**, 878.

G. ACTION OF ELECTRICITY AND OF OZONE

The passage of a continuous current of a few milliamperes through a solution of ammoniacal salts or of amino bodies containing tubercle bacilli in suspension has the effect of attracting the organisms in mass to the cathode. Ch. Russ[14] thought to utilize this property for the detection of bacilli in suspected fluids where the bacilli were too few to be revealed by direct examination of slides. A little ethylamine is dissolved in the liquid (urine, milk, etc.), and the current passed through. Whatever gathers upon the cathode (the latter being enclosed in a glass tube) is collected, smeared and stained.

Dry air charged with ozone to a concentration of 4 to 6 mgms. per liter, destroys the vitality of the bacillus within a few minutes (Marmier and Abraham).

H. EFFECT OF AGE UPON CULTURES

Long ago laboratory experience showed that the virulence of a tubercle bacillus culture falls off rather rapidly if the organisms are not frequently replanted upon new media. As a general rule the resowings should be made at least every month. The cultures, after being left in the incubator at 38°C. for 6 to 8 weeks, have already lost a large part of their vitality. Virulence persists for a variable period according to the source of the bacillus. Those of avian origin are more stable than bovine, which in turn are more stable than human bacilli. The susceptibility of the latter is probably due to the fact that they produce more acid (Th. Smith). Old cultures, of 6 or more months, reproduce themselves only exceptionally. However they can still infect susceptible animals, although the development of lesions may be very slow. In general, after 8 to 10 months, almost all the bacterial elements are either dead or so devitalized that they can no longer be revived.

By regularly reinoculating a very virulent culture of bovine origin every three or four weeks on glycerin potato medium, we have preserved it unaltered for more than 15 years. The same dose of this culture (3 mgm.), suspended and injected intravenously into calves aged from 6 to 12 months, produces constantly an acute miliary infection with death in 28 to 35 days.

The solid culture media, particularly glycerin potato, sustain the

[14] Brit. J. Tuberc., 1910, **5**, 26.

virulence of the bacillus much better than do the fluid media. This is especially true if care is taken to remove the solid media cultures from the incubator after four weeks and to keep them in the cold.

I. ACTION OF VARIOUS CHEMICAL AGENTS

The more important chemical substances and the proportions which hinder or arrest the growth of pure cultures of the tubercle bacillus are: 1 in 100 potassium iodide; 1 in 900 arsenious acid, 1 in 900 boric acid, and the ammonia vapors (Villemin).

Carbolic acid in 5 per cent strength kills the bacillus in 5 minutes; the same acid in 1 per cent concentration kills it almost as quickly; corrosive sublimate, 1 to 100 kills in one hour, 1 to 1,000 in 24 hours.

Many other antiseptics have a bactericidal action upon the tubercle bacillus; for example 1 per cent tricresol; 2 per cent lysol; and formalin (1 per cent in one hour, 0.01 per cent in 24 hours). The same is true of several anilin dyes such as fuchsin, gentian violet, methylene blue and auramin. On the other hand iodoform, dusted upon culture media, hardly disturbs the growth and becomes inhibitive only when massive doses (up to 5 per cent) are added. Indeed every one knows that surgeons have long remarked the favorable effects of iodoform dressings in the treatment of tuberculous abscesses, despite the fact that experimentally this substance is totally devoid of efficacy. Baumgarten, Rovsing in Salomonsen's laboratory at Copenhagen, Troje and Tangl, Catrin, then Stchégoleff[15] under the direction of I. Straus, have brought many proofs of this fact, by mixing large quantities of iodoform either with cultures or with tuberculous products which were then inoculated into animals subcutaneously or intraperitoneally, or even into the anterior eye chamber of the rabbit, without the evolution of the infection being prevented; it was only, now and then, rather delayed. One must therefore conclude that iodoform acts by altering the tissues immediately about the tuberculous focus being treated, and not as an antiseptic.

Chloral in 1 per cent strength, oil of turpentine, benzin, terpin, creosote vapor, toluene and eucalyptus vapor scarcely retard culture growth (Villemin[16]).

The virulence of tuberculous products is destroyed only after 24

[15] Arch. de méd. expér., 1894, **6**, 813.

[16] Rev. de Verneuil, t. II, p. 237 ("*Étude expérimentale et clinique sur la tuberculose*").

to 36 hours by a 1 to 500 solution of salicylic acid; after two days by 8 to 10 times their weight of hydrogen peroxide (oxygen 12 volumes); or after two days by 1 to 1000 bromine water (Parrot and Hipp, Martin). Moreover the bacilli contained in mucous sputum, or in albuminous matter in general, are much more resistant to antiseptics than are those taken from cultures. They are killed beyond question only after at least 24 hours in 5 per cent carbolic solution or in a 1 to 1000 sublimate solution; after two hours in 2 per cent tricresol, in 4 per cent lysol, in 15 per cent formalin and in 2 per cent calcium chloride.

The sulphur dioxide set free by burning 60 grams of sulphur to the cubic meter kills in 24 hours of contact (Thoinot), but in 6 hours with the Clayton furnace with a concentration of at least 6 per cent in the air of the infected room (Calmette).

The most accurate work regarding the effect of different antiseptics on *avian* tubercle bacilli in pure culture is that of Yersin.[17] With a drawn out glass pipette he took up a little culture from the surface of a tube of glycerin agar of 15 days' growth and transferred it quickly into a test tube containing the antiseptic solution. After varying intervals of time he drew up a bit of the sediment in a pipette and put it into a test tube full of distilled water. A few hours later the material to be inoculated, well washed and freed from antiseptic, was transferred to a flask of glycerin broth kept ready in the incubator at 39°. Yersin then noted what flasks showed growth.

By this method the following results were obtained:

ANTISEPTICS	PROPORTION IN THOUSANDTHS	COMPLETE BACTERICIDAL ACTION
Carbolic acid	50	30 seconds
Carbolic acid	10	1 minute
Absolute alcohol	1000	5 minutes
Iodoform ether	10	5 minutes
Ether	1000	10 minutes
Bichloride of mercury	1	10 minutes
Thymol	3	2 hours
Water saturated with creosote	—	Incomplete
Water saturated with naphthol	—	Incomplete
Salicylic acid	2.5	6 hours
Boric acid	40	Incomplete after twelve hours

[17] Ann. de l'Inst. Pasteur, 1888, **2**, 60.

According to the studies of Moussu and Goupil,[18] of Uhlenhuth and Xylander,[19] C. Fraenkel and E. Baumann,[20] and F. M. Schmitt,[21] *chlorin*, in the form of Javel water or of antiformin, has a very pronounced action on the tubercle bacillus. Not only does the chlorin quickly destroy its virulence, but it also combines with its component elements to modify its original state. After the mixing, hydrochloric acid is found to be formed in appreciable amount. The bacilli, stained by the Ziehl method, no longer resist the action of dilute acids; acid-fastness has disappeared. By making numerous tests at successive intervals from the beginning of the action of the chlorin, one can detect the gradual diminution and finally the loss of acid-fastness. Since this quality, as will be seen later, persists despite the action of fat solvents and even survives the action of potassium solutions raised to the boiling point, it appears that chlorin therefore by itself produces a profound alteration in the constitution of the bacillus.

With *iodin*, the alteration is less marked. The bacilli treated with an excess of Lugol's solution for 15 to 30 minutes, then washed, centrifugated and taken up in physiological salt solution, are still toxic for cellular elements (J. Nicolau[22]).

Urea, when added to culture media in small proportions, has a definite inhibitive action, according to Rappin.[23] On the other hand, sodium chloride, even in proportions up to 15 or 25 per cent, is said not to affect the vitality of the bacillus. M. Muller (of Strassburg) proposed to utilize this property of salt to preserve *pathological material*, and also *milk* which was to be sent to the laboratory for examination for tubercle bacilli.

Certain *metals*, especially in the *colloidal state*, appear to have an unfavorable effect on the growth of the bacillus on nutritive media. Robert Koch[24] called attention long ago to the inhibitive effects of *gold cyanide* diluted to one in one million or even to one in two mil-

[18] Compt. rend. Acad. des sci., 1907, **145**, 1231.
[19] Arb. a. d. k. Gsndhtsamte, 1909, **32**, 158.
[20] Ztschr. f. Hyg., 1906, **54**, 247.
[21] Ztschr. f. Infektionskr. . . . . d. Haustiere, 1912, **11**, 321; 401.
[22] Compt. rend. Soc. de biol., 1914, **77**, 178.
[23] Ibid., 1901, **53**, 691.
[24] Internat. Congr. on Med., 10th, Berl., 1890.

lion parts. Bruck and Gluck,[25] A. Feldt,[26] Bettmann,[27] Junker,[28] Arthur Mayer,[29] L. Hauck,[30] and M. Breton,[31] have studied experimentally, as well as clinically, the effects of different gold preparations (*double cyanide of gold and potassium, colloidal gold, or arseniated colloidal gold* as prepared by Fourneau at the Pasteur Institute). Results were at times favorable, but more often negative.

The lack of stability of gold salts makes it impossible for them to penetrate into the organism without being decomposed. The same is true of *copper salts* (cyanide, sulphocyanide, sulphate, tribasic phosphate, acetate), which have been recently tried by different experimenters, especially by Finkler and the Countess Von Linden (of Bonn),[32] A. Strauss, Meisen, S. Pekanowich. This holds likewise for *colloidal preparations of platinum, silver, palladium, rhodium, selenium,* etc., studied by Paul Courmont and A. Dufourt.[33]

Rénon,[34] without any more success, has utilized the salts of *nickel, yttrium, zirconium,* as well as *nickel, silicon* and *ruthenium in colloidal state.* He has also tried certain *anilin dyes,* either pure or combined with iodin (*iodized methylene blue*).

In Rénon's hands too, *thorium* (nitrate, sulphate, chloride) *mesothorium,* and the bromide and sulphate of *radium* had no inhibitive action on cultures *in vitro,* nor any therapeutic activity experimentally.

A. Frouin has studied the influence of rare earths on the growth of tubercle bacilli. He finds that if, to the following medium, which is like that of Proskauer and Beck plus a little more lactose,

	gms.
Distilled water	1000.0
Asparagin	5.0
Lactose	3.0
Glycerin	40.0
Sodium citrate	1.5
Dipotassium phosphate	1.0
Magnesium sulphate	1.0

[25] München. med. Wchnschr., 1913, **60**, 57.
[26] Deutsch. med. Wchnschr., 1913, **39**, 549.
[27] München. med. Wchnschr., 1913, **60**, 798.
[28] München. med. Wchnschr., 1913, **60**, 1376.
[29] Deutsch. med. Wchnschr., 1913, **39**, 1678.
[30] München. med. Wchnschr., 1913, **60**, 1824.
[31] Compt. rend. Soc. de biol., 1913, **74**, 1200.
[32] Internat. Congr. on Tuberculosis, 10th, Rome, 1912.
[33] Compt. rend. Soc. de biol., 1913, **75**, 454.
[34] Rev. gén. de clin. et de thérap., 1903, **17**, 353; Compt. rend. de la Caisse nat. des recherches sci., 1913.

is added 0.4 gm. of *sodium vanadate* per liter, a much more abundant bacterial growth is obtained. The gain is less if ten times as much vanadate is added.[35]

The sulphates of *cerium, lanthanum, neodymium, praseodymium, samarium,* in doses of 0.05 gm. per liter of nutritive media, also favor the growth of tubercle bacilli. In a concentration of 1 to 1000 the sulphates of *neodymium* and *praseodymium* completely prevented growth. In any case these salts cannot replace magnesium in so far as the latter is necessary for growth.

V. Henri considers that the salts employed by Frouin act as catalysers and that, in large doses, they inhibit growth through the production of hydrogen peroxide or of other substances with very high oxidizing potential. This inhibiting action on the tubercle bacillus is lessened if easily oxidized bodies (glycerin, glucose) be added to the culture media.

Knowing well the very marked influence of the radio-active salts of *thorium* and of *uranium* on the germination of grains and the later development of their organs, F. Becquerel[36] thought that these same substances might have an analogous effect on tubercle bacilli. To settle the point he incorporated into the usual culture media variable quantities of solutions of *uranium* or *thorium* nitrate. He found that small doses, up to a maximum of 0.4 mgm. of the *uranium* salt or 0.04 mgm. of the *thorium* salt per cubic centimeter of liquid, affect favorably the rapidity and quantity of film growth. Beyond these doses, however, the toxic and inhibitive action becomes manifest.

A. Frouin,[37] who also investigated the same problem, did not secure exactly the same results. Working with *thorium sulphate* instead of the nitrate, and with *uranium acetate* he arrived at the conclusion that *uranium* does not favor the growth of tubercle bacilli, while *thorium* does favor it slightly.

[35] Compt. rend. Soc. de biol., 1912, **72**, 1034.
[36] Compt. rend. Acad. des sci., 1913, **156**, 164.
[37] Compt. rend. Soc. de biol., 1913, **74**, 282.

CHAPTER IV

CHEMICAL COMPOSITION OF THE TUBERCLE BACILLUS

The average weight of one tubercle bacillus is about 2.5 one hundredth millionths of a milligram.

There are approximately *40 million bacilli in 1 mgm. of growth* on artificial media, whether solid or fluid, when weighed in the fresh state after incomplete drying between double thicknesses of blotting paper.

According to Nebel,[1] *the specific gravity of the tubercle bacillus varies between 1010 and 1080.* The organisms have therefore a great tendency to fall to the bottom of culture fluids. They remain on the surface,—a condition essential for their growth on the majority of artificial media,—only when they exist in masses composed principally of young organisms. The waxy fatty ectoplasm which comprises in part each of the microörganisms prevents their becoming soaked and ensures their clumping together.

The water content of the bacilli has been determined by Hammerschlag[2] as averaging 85.9 per cent.

A. MINERAL COMPOSITION

After being extracted with alcohol and ether, dried at 100°C. and calcined, the bacilli yield about 8 per cent of ash, which, according to the analyses of Schweinitz and Marion Dorset[3] contains per 100 parts:

Sodium	13.62
Potassium	6.35
Calcium	12.64
Magnesium	11.55
Silicon	0.57
Phosphoric acid	55.23

The absence of chlorin and sulphuric acid is probably due to the preliminary washing in hot water. Furthermore these figures are only approximate, since the chemical composition of the bacilli varies somewhat with their origin and the artificial culture media.

[1] Arch. f. Hyg., 1903, **47**, 57.

[2] Centralbl. f. klin. Med., 1891, **12**, 9.

[3] Centralbl. f. Bakt., Ref., 1903, **33**, 278.

whether solid or fluid, from which they are taken. This explains why the same investigators have found the proportion of phosphoric acid per 100 parts of ash to be as follows:

For bovine bacilli	58.04
For bacilli of swine	56.48
For bacilli of the horse	55.40
For avian bacilli	55.63
For attenuated human bacilli	74.38
For virulent human bacilli	60.90

The phosphorus content parallels the variations in fats. The proportion of total ash to the weight of fresh bacilli varies between 2.31 and 3.96 per cent. Hammerschlag gives 2.55 per cent.

Kressling gives the following composition for bacilli dried at 100° to 110°C.

	per cent
Albuminoid substances	53.59
Fatty substances	38.95
Non-nitrogenous substances (by subtraction)	0.972
Nitrogen	8.575
Ash	2.55

Von Behring[4] cites the results of analyses of bovine bacilli by Zincke. Ash content was to the weight of *dry* bacilli as 6.91–7.3 to 100. Combined calcium and magnesium phosphates made up 4.2 per cent. The insoluble fraction contained some carbonate and some phosphate of calcium, some phosphate of magnesium and traces of iron too small to be calculated.

Siebert[5] has made a comparative study of the ash content of peptonized meat broth without glycerin and of bacilli cultivated on this same broth to which glycerin was added. The results obtained were as follows:

	ASH OF THE BROTH TOTAL ASH: 28.26 PER CENT OF THE DRY RESIDUE	ASH OF THE BACILLI TOTAL ASH: 7.52 PER CENT OF THE BACILLI DRIED AT 110°C.
Chlorin	43.98	6.60
Phosphoric acid	8.57	51.25
Sulphuric acid	2.25	0.84
Silicic acid	0.26	0.19
Sodium	29.69	9.18
Potassium	14.69	26.55
Magnesium	0.41	3.22
Calcium	0.15	2.17

[4] Behringwerke Mitt., 1907.

[5] Centralbl. f. Bakt., 1909, **51**, 305.

Bouveault[6] also has made comparative analyses of glycerin broth before and after growth of avian bacilli. He found that the latter assimilates more readily the simplest nitrogen bodies, those approaching the amins and ammonia, while sarcosin and the complex albuminoid compounds, such as gelatin and peptone, are recovered almost intact. As non-nitrogenous food they consume chiefly glycerin. The various sugars are scarcely touched.

B. SUBSTANCES EXTRACTIBLE BY FAT AND WAX SOLVENTS

Of all known bacteria, the tubercle bacillus yields the largest quantity of fatty and waxy bodies. To them, in large degree, it owes the property of acid-fastness and its chief biological characteristics.

These substances, very complex in constitution, are difficult to extract completely, a fact which explains the variable results in the analyses made by those who have undertaken their study. The proportion of fats and waxes is considerably influenced, moreover, by the age of the cultures, the origin of the bacilli and the various media used for cultivation.

Hammerschlag[7] estimates that the substances extractible by fat solvents represent on the average *27.2 per cent of the weight of the dry bacilli.* Aronson[8] puts the proportion at 20 to 25 per cent, Giaxa[9] at 35.2 to 40.4 per cent; Levene[10] at 31.56 per cent; Krebs[11] at 22 per cent; Von Schweinitz and Dorset at 37 to 42 per cent; Kressling[12] at 25 to 40 per cent; Ruppel[13] at 8 to 26.5 per cent; Baudran at 36 to 44 per cent; and Nicolle and Alilaire at 39 per cent.

It should be remarked that for extraction some of the investigators employed alcohol and ether, while others made use of ether, or alcohol and chloroform; and still others added benzol. Aronson was the first to use a mixture of ether, alcohol and hydrochloric acid, then recently trichlorethylene, the bacilli being treated in a shaking apparatus at a temperature of 37°C.

[6] Thèse, Paris, 1892.
[7] Centralbl. f. klin. Med., 1891, **12**, 9.
[8] Berl. klin. Wchnschr., 1898, **35**, 484; 1910, **47**, 1617.
[9] Centralbl. f. Bakt., Ref., 1901, **30**, 670.
[10] J. Med. Research, 1901, **6**, 135.
[11] Centralbl. f. Bakt., 1896, **20**, 488.
[12] Ibid., Ref., 1901, **30**, 1200.
[13] Ztschr. f. physiol. Chem., 1898, **26**, 218.

E. Roux and A. Borrel, at the Pasteur Institute, extracted the fats from bacilli by first boiling them in a weak solution of hydrochloric acid, then drying and treating them with hot xylol in an extraction apparatus. The preliminary treatment with acid can be replaced by heating in a drying oven at 140 to 150°C. The bacilli subjected to extraction with boiling xylol lose completely their property of acid-fastness. The total fatty substance, when so extracted and then spread upon slides, stains very intensely by *Ziehl* and is acid-fast.

Auclair and Paris[14] remove the fatty and waxy substance from the tubercle bacillus by treating successively with alcohol, ether and chloroform, allowing each solvent to act four days at 35°C. The alcohol dissolves out the stainable matter, a lipoid analogous to lecithin, some fatty acids and some alkaloidal substances yielding a chloroplatinate. The ether dissolves the neutral fats and a substance analogous to cholesterol, while the chloroform takes up a part of the latter substance and some waxy matter. Petroleum ether, in a Soxhlet apparatus or in a ball shaker, does not dissolve these products. From 8.18 gms. of dry bacteria, it dissolves 11.552 per cent; the alcohol acting afterward dissolves 5.708 per cent; the ether 14.975 per cent; and the chloroform 1.594 per cent; a total of 33.826 per cent of the whole bacterial mass. If the petroleum ether be dispensed with, the alcohol dissolves 17.260 per cent, the total of the first two fractions.

In the opinion of Auclair and Paris, acid-fastness is not a reaction limited to the waxy fatty matter; it persists in bacilli from which the fat has been removed and it belongs particularly to the cellulose framework and to the protoplasm of the microbe. The substance which holds together the clumps of bacteria (zooglea) is weakly acid-fast and gives the reactions of cellulose, since it resists boiling potassium and takes a blue color with iodine after treatment with sulphuric acid.

With hydrochloric alcohol or with benzaldehyde Deycke[15] extracted a neutral fat which he named *tuberculonastine*.

Von Schweinitz and Dorset saponified the fats, then distilled them in a medium acidified with sulphuric acid. In this manner they obtained some traces of volatile fatty acids and a larger quantity

[14] Compt. rend. Acad. des sci., 1907, **144**, 278.
[15] München. med. Wchnschr., 1910, **57**, 633.

of fixed fatty acids, some of them melting at 62°C. (those of palmitic acid); others with a melting point of 102°C. (those of arachidic acid); and finally others melting at 43°C. and which were identified as of lauric acid. According to G. Camus and Ph. Pagniez,[16] the property of acid-fastness is due to these fatty acids and they are formed only gradually by the bacillus, since young organisms show either no acid-fastness at all or but little.

In their opinion, tubercle bacilli, whether from cultures or from sputum, are more or less intensely stainable with blue, shading from a pale blue to a black, when subjected to the test proposed by Benda as specific for fatty acids. The material if fixed on the slide with heat, is treated with a hot saturated solution of sub-acetate of copper and washed generously with water, then put into a saturated solution of hematoxylin and differentiated with a very weak solution of potassium ferrocyanide and borax.[17]

Robert Koch and Proskauer found that the bacilli contain an acid substance insoluble in cold alcohol, but soluble in boiling alcohol and ether and which, in their opinion, is a non-saturated fatty acid. Aronson regards this as a true wax. He found that after treating this substance with boiling potassic alcohol, as for the saponification of waxes, there still remains an insoluble portion. This residue seems to be a higher fatty alcohol not identical with cholesterol and which stains with *Ziehl's* fuchsin. It is apparently a mixture of ceryl ($C_{27}H_{56}O$) and myricyl ($C_{30}H_{62}O$) alcohols.

According to Kressling,[18] the fatty mass contains 14.38 per cent of free fatty acids, 77.25 per cent of a mixture of neutral fats with higher fatty alcohols, and 8.37 per cent of water-soluble substances. Their melting point is about 46°C. Their ash contains a fairly large quantity of phosphoric acid derived particularly from lecithin. There is also some cholesterol. The higher fatty alcohols have a melting point of from 43.5 to 44°C. and represent 39.1 per cent of the total fats.

W. Bullock and J. R. Macleod[19] used boiling methyl alcohol to extract the fats from several kilograms of dried bacilli. On being left to cool, this extract threw down a whitish precipitate; this is the

[16] Presse Méd., 1907, i, 65.
[17] Compt. rend. Soc. de biol., 1905, **59**, 386; 701; 703.
[18] Centralbl. f. Bakt., 1901, **30**, 897.
[19] J. Hyg., 1904, **4**, 1.

acid-fast substance, which can be saponified and thus broken down into fatty acids and a snowy powder which chemical analysis shows to be an alcohol. They were able to obtain 1 gm. of it in a pure state. To this alcohol, in the last analysis, they attribute the acid-fast properties of the tubercle bacillus.

Dorset and Emery[20] also, separated from the non-saponifiable portion of the ether extract, an acid-fast alcohol which they thought belonged to the aliphatic series. Moreover Fontès[21] was able to extract some waxes by means of xylol, and among them he identified various alcohols; cholesterol, isocholesterol and phytosterin.

Aronson believes that the waxes are primarily a secretory product of the bacilli which unites them one to another, and that the bodies of the bacteria themselves do not contain any of it in their protoplasm.

W. T. Ritchie[22] thinks that the best solvents for the waxes of the tubercle bacillus are benzol with boiling for 48 hours, or benzol at ordinary temperature for 32 days or, still better, toluol with boiling for 8 hours.

The waxes, as I have been able to convince myself, are easily emulsified by heating gently near the melting point and rubbing up in an agate mortar (previously warmed in hot water) with a small quantity of egg yolk or beef bile, or simply with blood serum and physiological salt solution. The emulsion remains stable. It is agglutinated by neutral salts or by acids, as are the corresponding emulsions of beeswax.

White and Gammon[23] questioned whether any relationship existed between the fat of the tubercle bacillus and that of animals. It is known that the fat of adult man consists chiefly of palmitin and olein whereas that of the child is more rich in stearin, like that of cattle. These authors planted tubercle bacilli on 5 per cent glycerin agar in which they mixed, as thoroughly as possible, 6 to 20 per cent of various fats; control tubes were also made. The following facts were brought out: human fat and butter improve media designed for the human bacillus. On tubes to which human fat has been added, the growth is a hundred times more abundant than on ordinary glycerin agar. Linseed and olive oil are rather unfavorable. For the bovine

[20] Bur. Anim. Indust., Ann. Rep., Wash., 1904.
[21] Centralbl. f. Bakt., 1909, **49**, 317.
[22] J. Path. & Bact., 1905, **10**, 334.
[23] J. Med. Research, 1912, **26**, 257

bacillus, human fat, butter and olive oil are favorable, while linseed oil is rather inhibitive. Beef fat does not help either the human or the bovine bacillus. When the favorable fats are emulsified beforehand with an extract of liver, the advantage gained is said to be still greater.

C. CARBOHYDRATES

On treating the bodies of bacilli (previously freed of fat by alcohol and ether) with 1 per cent sodium hydroxide, and then again taking them up in concentrated sulphuric acid, a precipitate forms which dissolves in an alkaline cupric solution and which behaves like a cellulose in the opinion of Hammerschlag, like hemicellulose according to Dreyer and Marpmann, and Nishima. Auclair and Paris[24] regard it as a hydrocellulose, since it assumes a blue color with iodine solution. Bendix,[25] by treating the bacterial bodies with a hot 5 per cent hydrochloric acid solution, obtained a substance which gives with osazone the characteristic reaction of the pentoses. In his opinion, the pentoses are derived from the decomposition of the bacillary nucleoproteins.

Ruppel and Helbing regard the residue (about 8.3 per cent of the weight of the dry bacilli) obtained after treatment with strong mineral acids as a protein-like body analogous to keratin, chitin or to fibroin. Incidentally, the chitin which covers the eggs of the tenia gives the same color reactions as does the tubercle bacillus.

According to Baudran,[26] the bacillus contains a cellulose which, on being treated with equal parts of chloride of zinc and hydrochloric acid, is dissolved and gives the blue reaction with iodine. Permanganate at a temperature of 36°C. breaks it down into acetic and butyric acids. It is said to make up 3.6 to 5.5 per cent of the weight of the bacilli.

D. PROTEIN SUBSTANCES

The complete solubility of tubercle bacilli in certain chemical substances makes possible the study of their protein constitution. Robert Koch used concentrated alkalies for this purpose, but they are too strong and too destructive. Hammerschlag, used a 1 per cent

[24] Arch. de med. exper., 1907, **19**, 129; 1908, **20**, 737.
[25] Deutsch. med. Wchnschr., 1901, **27**, 18.
[26] Compt. rend. Acad. des sci., 1906, **142**, 657; 1910, **150**, 1200.

solution of potassium hydroxide. The solution of bacterial cells saturated with acetic acid and precipitated with ammonium sulphate yielded an albuminoid substance which reacts like the xanthoproteins with Millon's reagent, and gives a positive biuret test. Th. Weyl,[27] by means of acetic acid, separated a kind of mucin from an alkaline extract of bacilli which had been cultivated on glycerin agar; this substance he named *toxomucin*.

According to Klebs, the bacillus is made up for the most part of nuclein. If, after being rid of their fats with alcohol-ether, the bacillary bodies are treated with hydrochloric pepsin and then with alcohol a substance containing from 8 to 9 per cent of phosphorus is precipitated.

Ruppel[28] washes the bacilli with water, then treats them with dilute alkali and thus separates out the nucleoproteins. Baudran maccerates the bodies of the bacteria for 8 to 10 days in a solution of 1 per cent hydrochloric acid at 80°C. and finds that there is thus dissolved out an amount of albuminoid substances equal approximately to 50 or 60 per cent of the weight of the bacilli.

Auclair and Paris, after extracting the fats, treat the bacilli with concentrated acetic acid; they thus obtain a paranucleoalbumin which they call *bacillo-casein* and which has especially toxic properties. When injected under the skin of an animal, it produces within 24 hours a nodule having the anatomical characteristics of the early gray tubercle; the corresponding glands become swollen and, later develop visceral lesions which reproduce, in the lung particularly, the appearance of grey tuberculous pneumonia. As for its general poisonous effect, it induces in the guinea pig a rapid emaciation, a progressive anemia, then cachexia and death, the latter intervening after about three months and after a single injection (*see Chapter V*).

As early as 1897 Von Schweinitz and Dorset had isolated from cultures on fluid media, a crystalline substance soluble in ether, alcohol and water, and possessed of very characteristic necrotizing properties for the liver, when injected into guinea pigs. They regarded it as a non-saturated acid of the fatty series.

R. Koch[29] attempted to extract the bacterial substance by grinding. After the mass had been finely triturated in an agate mortar and sub-

[27] Deutsch. med. Wchnschr., 1891, **17**, 256.
[28] Ztschr. f. physiol. Chem., 1898, **26**, 218.
[29] Deutsch. med. Wchnschr., 1897, **23**, 209; 1901, **27**, 829.

jected to extraction with distilled water, it furnished a special poison (*tuberculin TR*) which Koch employed in the treatment of tuberculosis. This tuberculin will be studied later.

According to Ruppel,[30] the ground up bacilli on being extracted with water, furnish two distinct chemical substances: one of them, which can be precipitated by acetic acid, is a nucleoprotein which he called "*tuberculosamine.*" The other is a nucleic acid which might be called *tuberculinic acid.* The latter is rich in phosphorus, no longer gives the reaction of albuminoids, contains no sulphur and behaves like nucleic acids from other sources. Its phosphorus content is from 9.2 to 9.4 per cent. On boiling on a water bath it throws down nucleic bases (probably guanin, a little xanthin, and adenin) and a phosphorated acid, tuberculo-thyminic acid. Von Behring [31] regarded tuberculinic acid as the true tuberculous toxin to which tuberculin owes its characteristic effects. Kitashima[32] thought, to the contrary, that this effect is due to the thyminic acid group.

Levene[33] considers that the tubercle bacillus contains both free and combined nucleic acid. In order to obtain the free acid, he dries and pulverizes the bacilli and submits them to repeated extractions in solutions of 5 per cent sodium chloride and 5 per cent ammonium hydrochlorate. The extracts are then treated with picric acid and acidified with acetic acid. The precipitate collected by filtration, is washed with alcohol, redissolved in water and again precipitated with alcohol. In this manner there is obtained a perfectly white sediment which is taken up in water acidulated with acetic acid. When treated with a solution of copper chloride another precipitate forms which is washed with water until no more copper remains; with alcohol until it contains no more chlorine; and finally with ether after which it is dried *in vacuo* in the presence of sulphuric acid. Desiccation is carried out in an oven at 105°C. until its weight remains constant.

The residue, after treatment with the sodium chloride, is treated for two hours with a 4 per cent solution of sodium hydroxide, and then neutralized with acetic acid. An excess of picric acid is added and acetic acid is used to acidify. After filtering, alcohol is added to

[30] Ztschr. f. physiol. Chem., 1898, **26**, 218; Beitr. z. exp. Therap., 1900, H. 4, 88.

[31] Behringswerke Mitt., 1907.

[32] Centralbl. f. Bakt., Ref., 1903, **33**, 727.

[33] J. Med. Research, 1901. **6**, 135

the filtrate and a precipitate is formed which is redissolved and precipitated anew. This precipitate fails to give the biuret reaction and possesses all the properties of *nucleic acid.* When heated with mineral acids it does not reduce Fehling's solution. By dissolving in water with a little alcohol and acidifying the solution with acetic acid a cuprous salt of nucleic acid is obtained.

By this method, starting with different cultural strains, Levene has obtained nucleic acids in which the phosphorus content varies from 6.58 to 13.9 per cent.

V. C. Vaughan and S. M. Wheeler used an alcoholic soda solution and extracted two substances from fat-free tubercle bacilli: one of them, toxic and soluble in alcohol; the other non-toxic and insoluble in alcohol. The toxic portion kills guinea pigs in doses of 75 to 100 mgm.

The action of *hydrogen peroxide* was studied by Mme. Sieber-Choumov,[34] because of its property of decomposing and reducing bodies of high molecular weight such as keratin and animal and vegetable pigments. This author found that heating at 143°C under a pressure of three atmospheres permits hydrogen peroxide to completely dissolve bacilli previously dried and rid of fat. For one gram of bacilli it is necessary to use 300 to 350 cc. of hydrogen peroxide (oxygen 15 volumes) and to keep the mixture in an autoclave for about 2 hours. Such treatment gives a colorless liquid without residue and apparently of no toxicity.

Much and Deycke[35] completely dissolve the bacilli by means of *cholin* and *neurin*, and without previously extracting their fat. *Lecithin* and other lipoids (*alkaline oleates*) can also be utilized for this purpose. Ditthorn noted that this dissolving process is very slow at a temperature of 15 to 20°C., but it is almost complete in four days at 57°C., provided one uses concentrated solutions of neurin (25 per cent solution, Merck). Neurin is a strongly alkaline substance requiring 22 cc. of a decinormal solution or 1 cc. of an 8 per cent solution of hydrochloric acid to completely neutralize 1 cc.

Salimbeni[36] had better results with certain glycerin ethers, especially with *monchlorhydrin.* This solvent, which is soluble in water, enables one to treat the bacilli directly and without previously drying. With

[34] Compt. rend. Soc. de biol., 1913, **74**, 478.
[35] München. med. Wchnschr., 1909, **56**, 1825.
[36] Compt. rend. Acad. des sci., 1912, **155**, 368.

it one can observe a series of modifications, more or less rapid and profound, according to the proportion between the bacterial mass, the ethers employed and the number of their acid radicals.

If equal parts of bacilli and *mono* or *dichlorhydrin* (the latter is but slightly soluble in water) are rubbed up in a mortar, the bacterial mass becomes converted in a few seconds into an oily, homogeneous, more or less sticky paste. If a few more drops of reagent be added the paste is transformed into a very turbid fluid which clears itself more and more as the proportion of glyceride is increased. When *trichlorhydrin* is allowed to act upon the dried, finely-ground bacteria, a very fine emulsion is immediately obtained which is more transparent than those prepared with the *mono* and *dichlorhydrin.* Taking everything into account, the rapidity of the action, the appearance of the emulsions and the fineness and transparency of the bacterial clumps in suspension in the liquid, it is evident that the action of trichlorhydrin on tubercle bacilli is stronger and more complete than that of dichlorhydrin, and that the latter in turn is more active than the mono compound.

When treated with trichlorhydrin, the bacilli lose their acid-fastness in a few minutes, become granular and are transformed into an amorphous mass which no longer takes the stain. If treated with water, this matter gives up a fairly large quantity of a soluble substance which can be precipitated by 3 volumes of absolute alcohol. The water-insoluble residue contains the nitrogenous elements of the bacteria, along with the fats and the waxes taken up by the glyceride water which had dissolved them. It gives the characteristic reactions of nitrogen and of albuminoid substances. In the water-soluble portion, on the contrary, no trace of nitrogenous substance can be demonstrated.

In addition to this remarkable dissolving action, the glycerin ethers possess, for the tubercle bacillus, a bactericidal action so strong that only a few seconds of contact suffice to kill the microörganisms and to render them, after washing with water, innocuous for the guinea pig.

It is seen then that our knowledge of the chemical composition of of the tubercle bacillus, although incomplete, is nevertheless sufficient to permit of the extraction of the essential elements, so that the study of the physiological action of each of them becomes possible.

CHAPTER V

TOXINS OF THE TUBERCLE BACILLUS.—EXO- AND ENDOTOXINS.—TUBERCULINS

Tubercle bacilli contain toxic substances which are set free by prolonged maceration or dissociation of the bacterial elements, whether by grinding or by the aid of certain solvents (such as *caustic alkalies, neurin, mono, di or trichlorhydrin*). These toxic substances are closely connected with the protoplasm. They are altered, but not destroyed by boiling. When injected into normal animals, they cause the formation of smaller or larger abscesses, according to the dose inoculated. In doses sufficiently large, they produce a slow intoxication which can terminate in cachexia and in death.

A. TOXICITY OF DEAD TUBERCLE BACILLI—ENDOTUBERCULINS—VOLATILE POISONS OF THE TUBERCLE BACILLUS

Whole bacilli, killed by heat, possess the same properties as the bacillary extracts. This fact was first demonstrated by Maffucci.[1] By inoculating eggs with killed cultures of avian tubercle bacilli and then incubating, this author obtained chicks which were cachectic, but were free from tuberculosis. He also observed that if he injected dead cultures of human tubercle bacilli subcutaneously into guinea pigs, an abscess was formed at the point of inoculation and the animals died in a state of cachexia after a period varying from 15 days to 6 months, according to the quantity inoculated. At autopsy he found lesions of atrophic sclerosis of the liver and of the spleen, but no tubercles. The same lesions and death by cachexia could be produced by feeding guinea pigs with killed cultures. The accuracy of these facts has since been verified.[2]

A little later, Prudden and E. Hodenpyl published the results of some interesting experiments showing that dead bacilli when introduced in capillary tubes under the skin of rabbits, exerted a positive chemotactic influence on the leucocytes, and that the same dead

[1] Centralbl. f. allgem. Path. u. Path. Anat., 1890, **1**, 825.

[2] Compt. rend. Acad. des sci., 1906, **142**, 441.

bacilli, injected intravenously, provoked the formation of nodules which apparently had all the characteristics of tubercles in the lungs, but which did not go on to caseation. On microscopic examination stainable tubercle bacilli were readily demonstrated in the lesions.

By injecting dead bacilli directly into the trachea, the same authors demonstrated the formation of foci of hepatization characterized by masses of leucocytes and epithelioid cells with giant cells. The foci never became caseous and ended by being absorbed after encapsulation with fibrous tissue.

I. Straus and Gamaleia[3] carried out a similar series of investigations utilizing cultures of the human bacillus killed at 115°C. in an autoclave. They likewise observed the formation of real miliary granulomata in the rabbit lung after intravenous injection and in the peritoneum after intraperitoneal injection. On injecting a heavy suspension of the bacillus subcutaneously into the rabbit, an abscess was produced, which opened spontaneously after several weeks and discharged a creamy pus: but the corresponding lymph nodes showed no tumefaction, contrary to what is always found after subcutaneous inoculation of living bacilli.

Cultures heated at 130°C. for one hour during each of ten successive days, or submitted to several prolonged boilings in absolute alcohol, retain the same properties. This is also true of bacilli from which the fats have been removed with methyl alcohol and petroleum ether (Cantacuzène).[4]

The fundamental characteristic of the lesions produced by these dead bacilli, in animals free from any pre-existing tuberculous infection, is that they remain localized at the points where the bacilli were deposited in the body; they never become generalized. These lesions of localized tuberculosis are evidently due to intra-cellular poisons; that is to endotoxins of the tubercle bacillus.

Grancher and Ledoux-Lebard,[5] Vissmann,[6] Kostenitsch,[7] Masur and Kockel,[8] Krompecher, Kelber, Engelhardt, and Baumgarten obtained similar results, both with avian bacilli and with cultures killed and stained with fuchsin.

[3] Arch. de méd. expér. et d'anat. path., 1891, **3**, 705.
[4] Ann. de l'Inst. Pasteur, 1905, **19**, 699.
[5] Arch. de méd. expér., 1892, **4**, 1.
[6] Virchow's Arch., 1892, **129**, 163.
[7] Arch. de méd. expér., 1893, **5**, 1.
[8] Ann. de l'Inst. Pasteur, 1900, **14**, 723.

Kossel, and Weber and Heuss[9] killed human bacilli with heat and caused them to be inhaled by the ox. They observed that, although this animal is not susceptible to the same bacillus alive, it reacts nevertheless to the endotoxin by forming local pulmonary lesions. To this tuberculous endotoxin Von Behring[10] gives the name of *Somatine.*

Protoplasmic endotoxins

Auclair and Paris[11] believe that they have isolated the true endotuberculin formed by the protoplasm of the bacillus. This substance behaves as a *paranucleoalbumin* and its chemical properties are indistinguishable from those of casein. They have named it *bacillocasein.* Injected subcutaneously, it produces within 24 hours a nodule similar to the gray granulation; the corresponding glands become swollen and, some time afterward, visceral lesions appear which, in the lung particularly, simulate gray tuberculous pneumonia. In addition there is caused a rapid emaciation, a progressive anemia, cachexia and death. With a single dose death occurs in about 3 months.

The technique employed by Auclair and Paris to separate the different protoplasmic poisons from the bacilli was as follows:

The bacteria are first washed in water and then allowed to macerate in distilled water for 24 hours in an incubator at 38°C. The filtrate now contains a very small quantity of albumins and some albumoses. The albumins are precipitated with difficulty by a saturated solution of ammonium sulphate; the albumoses remain in the liquid. The bacilli are next left to macerate under the same conditions and for the same period of time in a 1 per cent solution of sodium chloride. The filtrate then contains traces of globulins which are precipitated by saturated magnesium sulphate and collected by dialysis. The above steps must be conducted aseptically.

These three classes of substances constitute the *soluble poisons* of the bacillus. When heated on a water bath for several hours, they possess the properties of *tuberculin.* We have here the T. V. of von Behring.

The bacilli after having been dried in the air are placed in the

[9] Tuberk.-Arb. a. d. k. Gsndhtsamte, 1905, H. 3, 30.

[10] Behringswerke Mitt., 1907.

[11] Arch. de méd. expér., 1908, **20**, 737; Bull. Soc. d'étude scient. de la tuberc., 1911/12, 2. s., 22.

exhausting chamber of a digestor with automatic intermittent siphonage, and extracted with *absolute alcohol.*

There are collected:

1. A coloring matter;
2. An alkaloid extracted with phosphotungstic reagent;
3. Some fatty acids extracted with a solution of sodium carbonate;
4. A little lecithin, extracted with acetone or cadmium chloride in alcoholic solution, and recovered later by treatment with hydrogen sulphide.

The extraction continued with *ether* yields:

1. Some neutral fats;
2. A large quantity of lecithin;
3. A little cholesterol.

The lecithin is precipitated with cadmium chloride and the cholesterol is isolated by crystallization.

Further extraction with chloroform yields:

1. A substantial quantity of cholesterol;
2. Some waxy substances, undetermined and small in amount.

The chloroform solution is evaporated to dryness and then again taken up in *boiling alcohol-ether.*

On cooling, the cholesterol crystallizes out in long glistening needles.

All of the extractions are carried out *in vacuo* in order to lower the boiling point of the solvents and to avoid alteration of the extracted products by too high a temperature.

These substances make up the *lipoid or adipowaxy poisons* of the bacillus (*ethero-bacilline* and *chloroformo-bacilline*).

The bacillary mass is treated at a temperature of 80°C. with pure concentrated acetic acid which dissolves the caseins without altering them.

The acetic solution is precipitated with sodium hydroxide.

The precipitate washed in water, alcohol and ether and dried in a vacuum, constitutes the *bacillo-casein.* After washing in 90 per cent alcohol it can be preserved in the fresh state in emulsion without glycerin. It loses some of its properties with time.

The bacillary mass so treated contains only a small quantity of *nuclein,* soluble only in caustic potash which modifies its constitution, and of *cellulose* which can be recognized by its characteristic reactions (quick transformation into hydro-cellulose with sulphuric acid and a later blueing with Lugol's solution).

The chemical and biological characteristics of the different toxins to be extracted from the tubercle bacillus are tabulated by Auclair and Paris:

TOXINS	EXTRACTING FLUIDS	CHEMICAL PROPERTIES	BIOLOGICAL PROPERTIES
First class: *Soluble toxins*	Water	Albumoses, Albumins (Traces of nucleoproteids)	Not well understood at present
	NaCl solution 1 per cent	Globulins (Traces of nucleoproteids)	
Second class: *Lipoids*	Alcohol	Fatty acids, Alkaloids, Lecithin	*Local action:* Caseation
	Ether	Neutral acids, Lecithin, Cholesterol	
	Chloroform	Cholesterol, Waxes	*Local action:* Sclerosis
Third class: *Protoplasmic toxins*	Acetic acid; Neutral salts of alkaline reaction	Paranucleo-albumin (bacillo-casein)	*Local action:* Nodules, glands, small visceral tubercles. *General action:* Congestion, hematopoietic disturbances, cachexia, death

Volatile bacillary poisons

In addition to the extractible and stable poisons, the tubercle bacillus produces certain others of a volatile nature. They are well known to bacteriologists who have experienced their effects in evaporating cultures on a water-bath for the preparation of tuberculins, or in grinding dry tubercle bacilli killed by heat.

Auclair called attention to them in his thesis and Armand-Delille[12] describes very minutely the train of symptoms which he had the

[12] Bull. Soc. d'ètude scient. de la tuberc., 1913, Dec.

opportunity of observing in his own case. There is a sensation of muscular pain, at first lumbar, then general, coming on 6 to 8 hours after the manipulation of the bacilli. Then there are one or more severe chills, with nausea and violent headache. The temperature gradually rises to about 39°C. After a period of restless sleep lasting 4 to 5 hours, sweating sets in, the fever falls and there remains only a general lassitude of varying duration.

Bacteriologists are not all sensitive to these volatile poisons of the cultures, but those who are susceptible and who suffer brisk reactions do not appear able to immunize themselves by habituation. Moreover they also react to tuberculin.

B. TUBERCULIN OF ROBERT KOCH—ITS PREPARATION

Comparative study of lesions produced by dead tubercle bacilli in healthy animals and in those already rendered tuberculous, led Robert Koch to the discovery of *tuberculin*.[13] He had noted that the effects produced by subcutaneous injection of dead bacilli are quite different in the infected body. Healthy guinea pigs tolerate large doses of the dead bacilli, reacting with a simple local abscess, while tuberculous guinea pigs on the contrary are killed in 6 to 48 hours by very small quantities. If the dose is so small as not to lead to death, a more or less extensive area of cutaneous necrosis is produced about the point of inoculation; and if one continues to inject still smaller doses, the general condition of the tuberculous animal improves, the inoculation ulcers become cicatrized, the primarily engorged lymph nodes diminish in size and the evolution of the disease seems to undergo a period of arrest.

Thinking at once that this action was due to a toxic substance liberated by the dead bacilli, Robert Koch attempted its extraction. To this end he first scraped off cultures grown on glycerin agar and took up the material with a 4 per cent solution of glycerin in water: the mixture was evaporated on a water bath to one-tenth of its original volume and the bacilli removed by filtration.

But before long he adopted the technique which is still used in preparing what is today called Koch's *old tuberculin*. It was originally prepared as follows:

A liter of weakly alkaline beef broth, containing 10 grams of peptone and 40 to 50 gms. of glycerin, is poured into a large flat

[13] Deutsch. med. Wchnschr., 1891, **17**, 101.

bottom flask until the latter is about one-third full. This is sterilized in the autoclave at 120°C. and after cooling a fragment of culture is inoculated upon the surface of the medium. The flask is put away in the incubator at 38°C. After 6 to 8 weeks, when the completely developed film tends to break up on the surface of the liquid, the latter is evaporated on a water-bath to one-tenth of its original volume and filtered through a porous earth (*Berkefeld*) or porcelain (*Chamberland*) filter. The tuberculin so obtained is clear, syrupy and of a dark brown color.

In most laboratories, the original technique has been improved. The cultures are first sterilized with steam at 100°C. and then evaporated on the water-bath; the concentrated liquid is filtered through two layers of thick paper (Chardin paper), which retain most of the bacterial bodies.

A dose of 0.1 to 0.3 cc. of this tuberculin should kill within 6 to 24 hours guinea pigs injected subcutaneously four weeks before with one centigram of a culture of tubercle bacilli, a quantity ordinarily sufficient to cause the death of the animals in 8 to 10 weeks. On autopsying the guinea pigs there is found, at the point of inoculation of the bacilli and round about it, a red edematous infiltration which extends to the corresponding lymph nodes. The spleen, liver, lungs and small intestines show small, dark-red ecchymotic spots due to extravasations of blood. The suprarenal capsules are increased in volume and congested.

Von Bergman in 1890, in a case of tumor of the cheek, was the first to use tuberculin as a diagnostic agent in man.

C. EXO- AND ENDOTOXINS OF THE BACILLUS

The original tuberculin of Robert Koch contains both the exotoxins or soluble poisons excreted by the bacillus into the culture media, and a portion of the protoplasmic endobacillary poisons. The diffusion of the latter into the liquid is facilitated by the slow concentration with heat, the media becoming richer and richer in glycerin. The exotoxins however are particularly abundant since, on the one hand, the tubercle bacilli retained on the filter after maceration are scarcely less toxic than if they had simply been killed by heating during an equal period. On the other hand, a tuberculin,—definitely less toxic to be sure but still active,—can be prepared by concentrating the culture media from which the tubercle bacilli have previously

been separated. As a matter of fact it is in this way that Denys (of Louvain) prepares his tuberculin for treating patients. It is nothing but culture broth filtered through porous candles.

Maragliano[14] evaporates this same filtered broth at 30°C. in a vacuum and obtains a liquid of which 1 cc. causes a fall of temperature and kills healthy guinea pigs. This liquid, contrary to what occurs in the case of Koch's tuberculin and the bacilli, is said to lose its toxicity on being heated to 100°C. (but this has been shown to be incorrect by A. Koeppen).[15] Maragliano considers it the *toxalbumin* of the cultures, as distinct from the *toxoproteins* of the bacilli.

By growing the bacilli on a liver broth to which is added glycerin and a leucotoxic serum, which he secures from animals several times inoculated with emulsions of spleen or of leucocytes, Marmoreck[16] prepares a soluble toxin which, after simple filtration, is more toxic for healthy than for tuberculous animals. The subcutaneous injection of 5 to 10 cc. suffices to kill a normal guinea pig or rabbit. This soluble toxin is used to inoculate horses for the production of his anti-tuberculosis serum.

D. PURIFIED TUBERCULINS

Koch himself and many later investigators have tried to purify the original tuberculin by eliminating substances contained in the culture media and which themselves might be harmful (peptone, salts, waxes and fats). The method most to be recommended consists in diluting a measured quantity of crude tuberculin in 5 parts of distilled water and pouring the mixture, a little at a time and with constant shaking, into 20 volumes of 95 per cent alcohol.

The precipitate is collected on a Berzelius paper filter, redissolved in a quantity of water equal to the original quantity of raw tuberculin and then precipitated a second time with 20 volumes of alcohol. The second precipitate, when dried in an oven, furnishes a spongy, grayish-white mass, soluble in water and very toxic. One milligram of the powder produces the same effects as 50 mgms. of raw tuberculin. The quantity obtained varies between 1 and 2 gms. per 100. When redissolved in 50 per cent glycerin water, this precipitated

[14] Berl. klin. Wchnschr., 1899, **36**, 385.
[15] Ztschr. f. Hyg., 1906, **52**, 111.
[16] Bull. Acad. de méd., 1903, **50**, 332; 465; 480.

tuberculin gives clear solutions, easily preserved and which can be sterilized in the autoclave at 120°C. and are very stable. But it still contains many impurities which can be eliminated in large part by repeatedly re-dissolving and re-precipitating.

More easily purified products are obtained by starting with cultures on liquid media without peptone, such as the one the composition of which I worked out with L. Massol (see Chapter II). Such a culture is concentrated in a vacuum at 45°C. and only to one-fifth of its original volume. The liquid is precipitated with 20 volumes of a mixture of equal parts of 95 per cent alcohol and sulphuric ether; the precipitate is re-dissolved in a small quantity of water, dialyzed on animal parchment for 6 to 32 hours in cold running distilled water, and precipitated a last time with 10 volumes of absolute alcohol. The white powder (*Tuberculin* CL) obtained is still 10 times more active than that furnished by precipitating old tuberculin with alcohol. The quantity obtained is about 0.75 gm. per liter of culture.

E. CHEMICAL PROPERTIES OF THE TUBERCULINS

Koch's tuberculin purified by precipitation with alcohol gives all the reactions of albuminoid substances. According to Kühne,[17] it contains some deutero-albumoses, a special albumose (acro-albumose), some peptones, some tryptophan, and an indol-like body. It is precipitated by ammonium sulphate, iron acetate and tannin; in part by lead acetate. Acetic acid produces first a considerable clouding and even a little sediment which is redissolved in an excess of the acid. Picric acid gives a flocculent precipitate which disappears on heating, to reappear again on cooling. Hydrochloric and sulphuric acids, both weak and concentrated, yield no precipitate.

Analysis of the ash has given the following results in the hands of various workers:

	WEIGHT OF SUBSTANCES DRIED AT 100°C.	WEIGHT OF ASH OBTAINED	PER 100
	grams	*grams*	*grams*
Brieger	0.4816	0.0802	16.65
Proskauer	0.1410	0.0265	18.46
———	0.1740	0.0350	20.46

[17] Ztschr. f. Biol., 1892, **29**, 26.

The ash is made up almost exclusively of potassium and magnesium phosphates. It contains no chlorides.

Elemental analysis of the substance without the ash, shows, according to Brieger:

	per cent		per cent
C	47.02	C	48.13
H	7.55	H	7.06
N	14.45	N	14.46
		S	1.17

and according to Proskauer:

	per cent		per cent
C	47.67	N	14.73
H	7.18	S	1.14

Ruppel[18] has studied the chemical composition of the bacillary endotoxin (bacilli ground and macerated in water). He found a *nuclein* decomposable into a *tuberculo-nucleic acid* and a *protamin* which crystallizes in small hexagonal plaques (*tuberculosin*) and of which one gram is said to have the same toxicity as 25 to 30 cc. of Koch's old tuberculin. According to Ruppel, all tuberculous toxin is made up essentially of *tuberculosin*, which is itself derived from the decomposition of a *tuberculothymic acid*.

But it is not at all certain that this tuberculosamin really represents the tuberculous toxin. Its high degree of toxicity is not a decisive argument, since other protamins, which can be extracted for example from the sperm of the sturgeon (Neufeld), are as toxic for the guinea pig, if inoculated intracerebrally, as is the tuberculosamin of Ruppel. Moreover the latter is not noticeably more toxic for tuberculous animals than for healthy animals. It is said that from 100 gms. of dry bacilli 8.5 gms. of tuberculonucleic acid, 24.5 gms. of nucleoprotamin and 23 gms. of nucleoproteins are to be derived.

André Jousset[19] concluded from his researches, chemical as well as biological, that tuberculin in broth cultures is associated neither with the albumins nor with the peptones, but with the lowest products of the culture medium, the *amino-acids*. The tuberculin appears, according to him, between the first and second week after

[18] Ztschr. f. physiol. Chem., 1898, **26**, 218; Beitr. z. exp. Therap., 1900, H. 4, 89.

[19] Bull. Acad. de méd., 1914, **71**, 752.

inoculation of the media and the increase parallels the growth of the film. It is a sort of waste product which can in no way be likened to genuine toxins.

The tuberculins prepared according to Koch's method (old tuberculin) have an alkaline reaction when made from cultures of bovine bacilli; the reaction is *acid* (to phenolphthalein) if made from human bacillus cultures (Th. Smith).

F. ESTIMATION OF THE TOXICITY OF TUBERCULINS

The toxicity of tuberculins for healthy animals can be measured by the method proposed by von Lingelsheim. It consists in trephining a guinea pig at a point approximately in the middle of a line which would join the posterior commissure of the two eyes (not exactly in the middle but a little to one side in order to avoid opening the superior longitudinal sinus). When the operation is well done there is no bleeding. The needle of the syringe is thrust perpendicularly into the cerebral mass of one or the other hemisphere to a depth of 3 to 4 mm. The injection is made gently so that all the liquid,—the volume of which should not exceed 0.5 cc.,—shall penetrate during 2 to 3 minutes. Glycerin solutions must not be employed in this inoculation procedure since glycerin itself is toxic for the nerve cells.

It is found that normal non-infected guinea pigs succumb in a few minutes to doses of 3 to 4 mgms. of precipitated old tuberculin, while doses 150 and even 200 times larger are innocuous if given by subcutaneous or intraperitoneal injection.

A. Borrel,[20] employing this method, has shown that the bacillary bodies washed and heated to 100°C. kill healthy guinea pigs, under the same conditions, in a dose of 0.5 mgm. In animals artificially infected 30 days before, 1/800 of this dose (0.01 mgm. of precipitated tuberculin) suffices, and even as little as 1/8000 (0.001 mgm. of precipitated tuberculin) is enough to kill guinea pigs infected more than 40 days before. The symptoms of intoxication are always the same (respiratory distress, convulsions, almost immediate asphyxia).

In Germany, the official titration of commercial tuberculin is regularly carried out by the Institute of Experimental Medicine at Frankfort, formerly under the direction of P. Ehrlich. The method of Otto[21] is used. It consists in taking, for example, 50 guinea pigs

[20] Compt. rend. Soc. de biol., 1900, **52**, 358.
[21] Arb. a. d. k. Inst. f. exper. Therap., 1906.

of the same weight (350 to 400 gms.) and injecting them intraperitoneally with 0.5 mgm. of a broth culture only 12 to 14 days old evenly suspended in physiological salt solution (1 cc. of liquid for 1 mgm. of the culture weighed in the fresh state). At the end of the third week 2 to 4 guinea pigs are autopsied to be certain that the tuberculosis is well developed and, if this is the case 2 to 4 others are tested out with 0.3 and 0.5 cc. of a standard tuberculin (*Standart-tuberkulin*). These doses should suffice to kill the animals when injected subcutaneously. If 0.5 cc. does not kill, it is because the tuberculosis is not sufficiently advanced and one must wait a few days before repeating. When the result is positive the guinea pigs are divided into two parallel series, one of which receives 0.05, 0.075, 0.1, 0.2, and 0.3 cc. of standard tuberculin, while the other receives corresponding doses of the tuberculin whose toxicity is to be measured. The death of the guinea pigs should occur in less than 24 hours and at autopsy there should be lesions characteristic of tuberculin intoxication. Under the above conditions the usually fatal dose of standard tuberculin is 0.075 cc.

Several investigators, particularly Detre and Spengler, thought that they found differences of toxicity in tuberculins according as they were prepared from cultures of *human* or *bovine* bacilli. Weber and Dieterlen[22] carried out many experiments in order to settle this question. Their conclusions agree entirely with those reached at the Pasteur Institute in Paris in 1891; namely, that the two tuberculins may be used indifferently on bovines and small laboratory animals, provided they have an equivalent toxicity measured, as has been said above, by the intracerebral method on tuberculous guinea pigs, which have been infected with the same quantity of human or bovine bacilli and have reached the same stage of the disease (about 4 to 5 weeks after the subcutaneous inoculation of one centigram of bacilli weighed in the fresh state).

Avian bacillus tuberculin has exactly the same relative action, but is always somewhat weaker.

A. Marie and Tiffeneau[23] have made an extensive study of the toxic action of purified tuberculins prepared from peptone-free media. With their product, made from unheated cultures, concentrated

[22] Tuberk.-Arb. a. d. k. Gsndhtsamte, 1910, H. 10., 217.

[23] Compt. rend. Soc. de biol., 1908, **64**, 501; 1909, **66**, 206.

in vacuo, dialyzed and precipitated, the rabbit succumbed after intracerebral injection of 0.02 gm. For the normal guinea pig the lethal dose was 0.00075 gm. (intracerebrally), and for the mouse 0.10 gm. (subcutaneously). The guinea pigs tuberculized four weeks before succumbed to 0.0001 gm. (intracerebrally).

One must therefore conclude that *the tuberculous poisons* (*endo and exotoxins*), *extremely toxic for animals infected with tuberculosis, are much less so,—although not entirely harmless,—for normal animals.*

The extraordinary sensitiveness to tuberculin on the part of tuberculous subjects, first noted by Koch, has been verified for all animal species, and this discovery has been the basis of innumerable applications (which we shall later discuss) to the diagnosis, prognosis and treatment of tuberculous infection.

We shall also consider in other chapters (XXXV, XXXVI and XXXVII) the physiological properties of the tuberculins, as well as their aptitude for serving as *antigens* and for generating "antibodies," or defensive substances, in healthy or tuberculous subjects.

For the moment we shall consider only how the tuberculins are prepared, their principal characteristics and the different products to be derived from them.

G. PRODUCTS DERIVED FROM THE TUBERCULIN OF KOCH

Beginning with Robert Koch, repeated efforts have been made to purify tuberculin. Unfortunately however a large number of preparations have also been made for commercial profit and have been offered to physicians and patients under various names, and to them the makers have attributed virtues for which there is too often no scientific basis. I shall here mention only such as are of some interest in research.

1. Tuberculin TR. Proposed by R. Koch[24] in 1897, this tuberculin is obtained by drying tubercle bacilli in a vacuum and finely grinding them in a mortar with an agate pestle. The bacilli, after prolonged trituration, are emulsified in distilled water and centrifuged for 30 to 45 minutes at 4000 revolutions. The supernatant opalescent fluid contains a part of the bacillary endotoxins and constitutes the preparation OT. The muddy residue, thrown to the bottom of the tube, is dried, ground, again taken up in water, centri-

[24] Deutsch. med. Wchnschr., 1897, **23**, 209.

fuged and reground several times until centrifugation no longer separates out intact bacilli. The different trituration residues are collected together and 20 per cent of glycerin added. We now have TR, which is an emulsion of the various substances comprising the bacterial cells. One cubic centimeter corresponds to about 2 mgms. of dried substance obtained from 10 mgms. of bacilli also dry. This tuberculin is manufactured at the Hoechst Chemical Manufactory, near Frankfurt on Main.

II. Tuberculin BE. In 1901, R. Koch[25] proposed to substitute a new preparation for the TR. It was presented under the name of *Neutuberkulin-Bazillenemulsion* or BE, and was obtained by making a suspension of either human or bovine bacilli previously dried and then very finely ground. The suspension is prepared by adding 1 part of the powder to 200 parts of distilled water glycerinated to 50 per cent and is not centrifuged. Dilutions are made with physiological salt solution, the strength being expressed in bacillus content and on the basis of one cubic centimeter of the initial raw product containing 5 mgms. of powdered bacilli.

III. Tuberculin AF (*Albumosefrei*) *of R. Koch.* This tuberculin is peculiar in that, unlike old tuberculin, it is made from cultures on media containing neither meat, nor peptones. Mineral media containing asparagin or ammoniacal salts are used for its preparation, that of Proskauer and Beck (modified by R. Koch) for example (see Chapter II). When the cultures have developed, which is approximately after two months, they are killed by a two hour heating at 60°C. on two successive days and are then filtered. The filtrate is evaporated in a vacuum to one-tenth of its original volume, and 0.5 per cent of carbolic acid is added for preservation. A number of guinea pigs are inoculated subcutaneously to be certain that no living bacilli remain. After this verification, the product is ready for use in the treatment of patients. It seems, according to those clinicians who have experimented with it, to be tolerated rather better (G. Jochmann and R. Moellers);[26] but the serum of patients who receive it is not perceptibly enriched in antibodies, and they continue to be sensitive to ordinary tuberculin, although they tolerate subsequent injections of bacillus emulsions in a better manner.

[25] Deutsch. med. Wchnschr., 1901, **27**, 829.

[26] Deutsch. med. Wchnschr., 1911, **37**, 1297; Veröffentl. d R. Koch Stift. z. Bekämpf. d Tuberk., 1912, II. 3, 29.

From the chemical point of view, this tuberculin, derived from an albumin free medium, gives the same albumin reactions, although less intensely, as that prepared with ordinary broth. *Therefore albuminous substances are formed during the growth,* and a large part of them are derived from autolysis of the bacilli.

In their investigations on albumin-free tuberculins, E. Löwenstein and E. Pick[27] found that their product did not show all of the characteristic reactions of albuminous substances. Their tuberculin, which is prepared from media having as a basis asparagin, ammonium lactate, sodium phosphate, sodium chloride and glycerin, is precipitated by alcohol, acetic tannin and by biniodid of mercury and potassium in a hydrochloric acid medium. The product is dialyzable and is destroyed by digestion with pepsin or trypsin. It has therefore rather the characteristics of a polypeptid.

IV. Tuberculocidine of Klebs. Klebs[28] treats tuberculin with alcohol, then redissolves the precipitate in water and treats the latter solution with a mixture of alcohol, chloroform and pure benzol. These solvents remove the impurities in large part. The product thus obtained is desiccated in an oven at 56°C. and again taken up in 100 cc. of glycerin containing 0.5 per cent of phenol. After a final filtration a substance is obtained which is soluble in alcohol, keeps indefinitely and which represents about 5 per cent of the tuberculin originally employed.

This preparation, according to Klebs, rid of the alkaloidal principles to which are due the harmful effects of the crude tuberculins, should have a peculiar effect on the bacillus which, in a sense become "vacuolized." Moreover it is only weakly toxic. Normal guinea pigs and rabbits tolerate 1 cc. without any discomfort and it is well borne by patients in doses 4 to 5 times larger than those of old tuberculin. Furthermore it is said to have the advantage of possibility of administration by mouth. Turban formerly used it in this way at Davos; his patients took a few drops in the morning on a fasting stomach, and there were some favorable results. Its use has, however, for a long time been discontinued.

V. Tuberculin of Maragliano. Even before Béranek, Maragliano[29] had proposed making use of the intra- and extracellular toxins to-

[27] Biochem. Ztschr., 1911, **31**, 142.

[28] Deutsch. med. Wchnschr., 1891, **17**, 1233.

[29] Berl. klin. Wchnschr., 1899, **36**, 385.

gether. His tuberculin is a mixture of *toxoproteins* derived from whole cultures macerated and concentrated at the boiling point, and of *toxalbumins* separated from the culture broths by concentration *in vacuo* at a low temperature. According to Bezançon and Gouget,[30] these toxalbumins tend to lower the temperature of both tuberculous and healthy guinea pigs. But A. Koeppen[31] has shown that there is in reality no difference between the two toxic substances from the point of view of their temperature lowering action on tuberculous guinea pigs. The active portion of the extracellular toxalbumins is thermostabile, as is that of the intracellular toxoproteins. Biochemical reactions are the same in the two groups, so that there is no advantage in separating them.

In 1898[32] Maragliano modified his method of preparation in the following manner:

The culture on glycerin peptone broth is filtered and the bacilli remaining on the filter are taken up in order to free them of all traces of glycerin. They are mixed with a quantity of distilled water equal to the original culture volume and the whole is left for 45 hours on the water-bath at 95 to 100°C., loss by evaporation being replaced. The mixture is afterward evaporated to one-tenth of its original volume and filtered. There is thus obtained an aqueous tuberculin of dark brown color and of alkaline reaction. Its effects are the same as those of Koch's old tuberculin.

Maragliano states that this watery tuberculin produces fever in both normal and tuberculous guinea pigs; normal guinea pigs are killed by a dose of 1 cc. per 100 gms. of body weight, while tuberculous pigs are killed within 48 hours by 0.1 to 0.2 per 100 gms. It is used chiefly in research, particularly for the preparation and titration of the therapeutic serum which Maragliano used in the treatment of patients at his clinic at Genoa (see Chapter XLI, 1).

VI. Oxytoxine of Hirschfelder. Hirschfelder (of San Francisco)[33] originally oxidized tuberculin by treating 40 gms. of Koch's raw product with 240 cc. of hydrogen peroxide (oxygen 10 volumes) and 936 parts of distilled water. The mixture was left for 96 hours in the autoclave. Later on he modified his procedure by adding the

[30] Compt. rend. Soc. de biol., 1899, **51**, 521.
[31] Ztschr. f. Hyg., 1906, **52**, 111.
[32] Compt. rend. Soc. de biol., 1898, **50**, 94.
[33] Lancet, 1898, i, 179.

peroxide directly to the glycerin veal broth culture in a proportion of one to ten. He sterilizes at 100°. Every twelve hours he adds a fresh quantity of hydrogen peroxide equal to the preceding, heats at 100°C. and continues thus until the volume of added peroxide is equal to the volume of the culture. He neutralizes with sodium hydroxide, adds 5 per cent of boric acid for preservation and filters. The *oxytuberculin* so prepared should contain no trace of free tuberculin nor of hydrogen peroxide. It is administered hypodermically, beginning with 5 cc. per day and increasing little by little up to 20 cc. Injections cause neither general nor local reactions; they are innocuous. L. Guinard and Mondielli[34] tried *oxytuberculin* upon animals and verified its non-toxicity. It does not appear to have been introduced into medical practice, nor have its therapeutic effects been well studied.

VII. Tuberculol of Landmann. Landmann[35] employed cultures whose virulence had been increased by successive passages through guinea pigs. When the cultures are well developed on glycerin peptone broth, the bacilli are collected by filtration. They are first rid of their fatty waxy envelope by appropriate solvents, then triturated and all their extractable products slowly removed at a temperature of 40°C. with physiological salt solution, distilled water and dilute glycerin. The supernatant fluid is poured off and the extraction repeated several times at gradually increasing temperatures, beginning at 50°C. and then through 60°, 70°, etc., up to 100°C. The extracts obtained at the different temperatures are combined and evaporated to dryness in a vacuum at 37°C.

The bulk of the endotoxins is thus collected, without perceptible loss and without alteration, since the bodies of the bacilli which remain are said to be no longer toxic.

These endotoxins are redissolved in the culture broth which is filtered and concentrated in a vacuum at 37°C. One cubic centimeter of the liquid so obtained is sufficient to kill a healthy guinea pig. Filtered through a bougie and supplemented with 0.5 per cent carbolic acid, it constitutes *Tuberculol.*

According to Landmann, the proof that this substance possesses properties distinct from those of tuberculin lies in the fact that its toxicity is much reduced by heating at 100°C., or even by prolonged

[34] Thèse, Lyon, 1898.
[35] Hyg. Rundschau 1898, No. 10; 1900, No. 8.

preservation in solution. It does not appear however that this toxicity for normal animals is really due to endotoxins derived from the bacilli since, according to the experiments of O. Bail and those of Löwenstein, one can inject 200 mgms. of living bacilli into guinea pigs, to the extent that their bodies are saturated with them, without any phenomena of intoxication being thus produced. The guinea pigs become tuberculous a little more quickly than those infected with only 2 mgms. of bacilli, and they die two or three weeks earlier. The toxicity of Landmann's preparation for healthy animals results perhaps from the fact that it contains decomposition products of the protoplasmic substances formed during the extraction at low temperature.

With tuberculous patients, treatment with this tuberculin should, according to its originator, be begun with a dose of 0.005 mgms. and be thence progressively increased up to 0.1 mgms.

Merck of Darmstadt supplies *tuberculol* in three forms, in order that clinicians may be enabled to utilize separately the endo and the exotoxins. *Tuberculol A* is the form just described; *tuberculol B* contains only the extractable products of the bacterial bodies; *tuberculol C* is made up by simply concentrating the culture media.

Tuberculols D, E, F, are also prepared, being derived from cultures of bovine bacilli, and are used for general or local tuberculin reactions. But all of these "specialties" are of no scientific interest.

VIII. Tuberculin of Béranek (of Neuchâtel in Switzerland). This tuberculin, prepared especially for tuberculin therapy, is used chiefly in Switzerland by Sahli (of Bern). It is a mixture of culture broth rid of bacilli by filtration through a *Chamberland* filter then concentrated in a vacuum at low temperature, together with an extract of the bodies of the bacilli obtained by macerating the latter for two hours at 60°C. in a 1 per cent solution of orthophosphoric acid which is afterward neutralized with sodium hydroxide. Thus is obtained AT (*acido-toxin*) or *endocellular toxin.*

The culture broth is prepared by macerating veal at room temperature, sterilizing it and then adding, 0.5 per cent of sodium chloride and 5.6 per cent of glycerin. It contains no added peptones.

The *tuberculin* of Béranek [36] is a 1 in 20 dilution of a mixture of

[36] Compt. rend. Acad. des sci., 1903, **137**, 889; Rev. méd. de la Suisse Rom., 1905, **25**, 684; 1906, **26**, 461; 1907, **27**, 444; Internat. Congr. on Tuberc., 6th., Wash., 1908.

equal parts of AT and of *filtered broth* (TB). For therapeutic use, there are prepared 15 *different solutions*, the concentration of which increases by multiples of 2, so that each solution is *twice as strong* as the preceding. It is not toxic for normal guinea pigs and is scarcely so for tuberculous pigs. According to K. Siegesmund its toxic power is 3.3 times less than that of the tuberculin control (*Standart-tuberkulin*) of the Institute of Experimental Medicine at Frankfurt, and it can be injected into tuberculous guinea pigs in amounts up to 16 cc. without the death of the animal.

IX. Tubolytin of Siebert and P. Römer. Siebert and P. Römer,[37] working in Von Behring's laboratory at Marburg, prepared a tuberculin without the use of heat or any chemical reagent which might modify or injure the active principle. They named it *Tubolytin.* This product, like Koch's old tuberculin, is innocuous for healthy animals and induces the characteristic reaction in the tuberculous animal. For the latter it is much less toxic, since it requires about five times as much tubolytin as tuberculin to cause death.

The two products, if inoculated intradermally, are equivalent.

The residue after evaporation (dry extract) is one hundred times, the ash content 39 times and the nitrogen content 43 times less than for old tuberculin.

The complement fixation reaction of Bordet-Gengou can be performed with 1 cc. of tubolytin and the presence of tuberculous antibodies be demonstrated in 0.0025 cc. of serum. To obtain the same fixation with old tuberculin, 0.02 cc. of the latter and 0.01 cc. of serum are said to be required.

X. Tuberculo-plasmin. E. Buchner[38] and Hahn[39] applied to tubercle bacilli a process devised by the former for extracting the zymase from beer yeast. They triturated the bacilli with sand, infusorial earth, 5 per cent of sodium chloride and 20 per cent of glycerin, and submitted them to a pressure of 400 to 500 atmospheres. In this manner they obtained a liquid of a light amber color, which is relatively stabile and is said to be a particularly active tuberculin. The difficulties of preparation are however so great that its use has never been extensive.

[37] Beitr. z. klin. d. Tuberk., 1913, **26**, 193.
[38] München. med. Wchnschr., 1897, **44**, 299.
[39] Ibid., 1897, **44**, 1344.

XI. Tuberculin of Rosenbach.[40] This tuberculin is a complex extract derived from a mixed culture of tubercle bacilli and of a mold, the *Trichophyton holosericum album.* It is prepared cold and carbolic acid is added for preservation. It possesses practically no toxicity, even for tuberculous animals which tolerate 5 cc. without difficulty. According to Lesser (Karl) and Koegel[41] this tuberculin is in no way superior to the old tuberculin of Koch; it appears to be about 1000 times less active than the latter and offers the disadvantage of containing non-specific products of the trichophyton.

It has been used in man in doses of 0.01 cc. to 0.1 cc. at the beginning of treatment and thence up to 2 cc.

H. Schaefer[42] considers that, under the influence of the proteolytic diastases of the *trichophyton,* the tuberculin of this product is simply digested and therefore becomes inactive.

XII. Tuberculin of Vaudremer. In studying the action of different microörganisms on the crude tuberculin of *Koch,* Vaudremer[43] observed that the proteolytic ferments, such as those of *B. pyocyaneus, Aspergillus niger, Aspergillus fumigatus* and *Penicillium glaucum,* destroy the active substance of tuberculin. He noted later that *Aspergillus fumigatus* renders living tubercle bacilli avirulent, and destroys tuberculin *in vitro* if the latter is submitted to prolonged maceration (24 hours at 39°) in the juice of ground-up mycelia. He thus obtains a tuberculin analogous to that of Rosenbach and, like the latter, almost devoid of toxicity, since 2 cc. of a 1 to 8 dilution are innocuous where 2 cc. of the same dilution of crude tuberculin in physiological salt solution would kill a tuberculous guinea pig within 24 hours. Vaudremer gives no information whatever as to the antigenic value of his preparation, so that the question arises as to whether the complete lack of toxicity on the part of his product (as is the case of Rosenbach's tuberculin) is not quite simply due to the fact that it no longer contains active tuberculin, the latter having been completely disintegrated by the thermostabile proteases of the mold, as is the case with trypsin and pepsin in artificial digestions, as we shall see later.

[40] Deutsch. med. Wchnschr., 1910, **36**, 1513; 1553;–1912, **38**, 539; 589.

[41] Beitr. z. klin. d. Tuberk., 1913, **27**, 103.

[42] Ztschr. f. Tuberk., 1911/12, **18**, 168.

[43] Ann. de l'Inst. Pasteur, 1910, **24**, 189; Compt. rend. Soc. de biol., 1912, **73**, 501; 1913, **74**, 278; 752.

XIII. Neurin-tuberculin of Much.[44] This is a solution of tubercle bacilli in *neurin*, a substance prepared by Liebreich and which is derived from the decomposition of brain tissue. Its chemical formula is said to be that of an hydroxide of trimethylethylammonium ($C_5H_{13}NO$).

Ten to 22 grams of bacilli are dissolved during 24 hours in 100 cc. of a 25 per cent solution of Merck's neurin. The bacterial cells at first become swollen, then the protoplasm disappears; but the granules continue unaffected for a long time. If heated to 56°C., only a few hours are required for solution.

The fatal dose of neurin-tuberculin for a healthy guinea pig of 300 gms. is about 0.1 gm. According to Wilhelm Schlaudraff,[45] of the Institute of Pathology of Saint Georges Hospital at Hamburg, the animals succumb with all the typical symptoms of neurin poisoning and the toxicity of neurin-tuberculin is no greater for tuberculous than for healthy animals. There exists therefore no hypersensitiveness to this product, from which one may conclude that neurin-tuberculin does not contain the specific substance to which the tuberculin reaction is due. It is said to be capable of binding antibodies and of serving as antigen for the complement fixation test; but repeated injections into animals (goats, rabbits, guinea pigs) are not followed by any antibody formation.

XIV. Tuberculo-mucin of Fr. Weleminsky.[46] If the film produced by the growth of human tubercle bacilli is frequently immersed in the culture media (glycerine peptone broth), two substances are gradually formed in the fluid. One has all the properties of a coagulable albumin; the other is a *mucin* occurring in fairly large amount and characterized by its mucilaginous appearance and by its being precipitated with acetic acid. This last permits of its isolation.

This *tuberculo-mucin*, a mixture of *proteins* and *mucin*, first dried and then redissolved in 100 times its volume of water, has been utilized clinically for the treatment of tuberculosis. Ernst Guth[47] begins with a dose of 1 mgm. (0.1 cc. of the solution) and increases gradually until a slight febrile reaction is obtained. It would seem to have some favorable effects, particularly in gland tuberculosis.

[44] München. med. Wchnschr., 1909, **56**, 1985; Centralbl. f. Bakt., 1910, **54**, 342; Berl. klin. Wchnschr., 1910, **47**, 1933.

[45] Ztschr. f. Immunitätsforsch., 1912, **12**, 91.

[46] Berl. klin. Wchnschr., 1912, **49**, 1320; Tuberculosis, 1914 **13**, 456.

[47] Ztschr. f. Tuberk., 1914, **21**, 554.

XV. Tuberculin bovine PTO (Perlsucht tuberkulin) of Spengler. This preparation is made exactly like the old tuberculin of Koch, except that bovine bacilli are used. Spengler,[48] who recommended it for a long time, believes that it is better tolerated and less toxic for man, and in a rather exaggerated manner he compares the "immunizing" effects to those of small pox vaccine.

As a matter of fact there is no difference whatever between the tuberculin prepared from bovine bacilli and that prepared from bacilli of human origin. The ideas put forth a few years ago by Detre and his pupil V. Gebhardt, relative to the possibility of determining the human or bovine origin of a tuberculous infection in man through differences in reaction to human or bovine tuberculins, were based upon errors of observation and are today abandoned.

Spengler himself seems to have given it up since employing his *immunizing bodies IK* which will be taken up in connection with the serotherapy of tuberculosis (Chapter XLI).

XVI. Tuberculous endotoxin of Baudran. Baudran[49] treats tubercle bacilli with 95 per cent alcohol which precipitates the albumins, peptones and albumoses, and dissolves the glycerin. He then filters and to the bacillary bodies adds successively ether, chloroform and toluene. Each solvent is eliminated after its action is completed.

The residue is treated with water, which readily dissolves the peptones and the albumoses, and then filtered through moist paper. The resulting liquid is concentrated in the presence of a few drops of a 1 per cent solution of sulphuric acid. The extract obtained is again taken up in alcohol, with which only albumoses and a small quantity of peptones are dissolved. He then neutralizes, filters, and gently evaporates. The substance obtained is completely soluble in cold water. It precipitates with saturated ammonium sulphate and acetic ferrocyanide of potassium; it takes on a red color with Millon's reagent and is not dialyzable.

This endotoxin, according to Baudran, is very toxic for the normal guinea pig which is killed by the intraperitoneal injection of 5 mgms. Such a fact would tend to indicate that it does not represent the true bacillary poison, but rather a modification resulting from the chemical reactions undergone.

[48] Deutsch. med. Wchnschr., 1904, **30**, 1129; 1905, **31**, 1228; 1353.
[49] Compt. rend. Acad. des sci., 1909, **149**, 941.

XVIII. Ferruginous tuberculin of Ditthorn and Schultz[50] (*Eisentuberkulin*). Ten cc. of old tuberculin are diluted in 50 cc. of sterile water and the whole precipitated with a 12 per cent solution of oxychloride of iron. The precipitate is collected upon a filter, washed for two days and redissolved upon the filter by adding a 1 per cent solution of sodium hydroxide, drop by drop. To this 25 per cent glycerin is added to make a volume of 40 cc., which are filtered and sterilized at 100°C. This product is *Eisentuberkulin A*.

An *Eisentuberkulin B* is prepared by utilizing the bacilli which have already served to produce the A preparation. They are washed several times in hot water and macerated for 24 hours in weakly carbolized water. The mixture is shaken from time to time to facilitate the extraction of the soluble substance. It is centrifugated and filtered, after which the filtrate is treated with 12 per cent oxychloride of iron as for the preparation of *Eisentuberkulin A*.

A third *Eisentuberkulin E* is made by beginning with unheated bacilli which, after being filtered from the culture broth, are washed with sterile water, then rapidly with alcohol to remove the water. The mass is next dried at 37°C. and extracted successively in a Soxhlet apparatus with ether and chloroform to remove the fats soluble in these solvents. The bacilli thus freed of their fats are left to macerate in water for 24 hours, and after centrifugation and filtration the solution is precipitated with oxychloride of iron. From here on the procedure is as for the preparation of Eisentuberkulin A.

Finally, a fourth *Eisentuberkulin S* is made from a broth culture 6 to 8 weeks old, from which the bacilli are filtered off as a preliminary step. The broth is concentrated to one-tenth of its original volume and then treated in the same manner as is old tuberculin in obtaining Eisentuberkulin A.

Fritz Ditthorn and Werner Schultz have likewise applied their method of precipitation with iron to cultures of bacilli grown on albumin-free media, such as that of Proskauer and Beck (asparagin 0.5; magnesium citrate 0.25; magnesium sulphate 0.06; monopotassium sulphate 0.5; glycerin 2 gms. per 100 cc. of water).

According to Schultz, subcutaneous injections of these substances rarely provoke any general reactions. They are but weak antigens and have no special practical virtue (Schellenberg).[51]

[50] Ztschr. f. Immunitätsforsch., 1909, **2**, 567; Deutsch. med. Wchnschr., 1908, **34**, 1221.

[51] Ztschr. f. Tuberk. 1911/12, **18**, 132.

XVIII. Tébéan of Levy and Kaenker. *Tébéan* is an emulsion of tubercle bacilli killed by being left for a long time in a 25 per cent solution of galactose at a temperature of 37°C. One gram of *tébéan* powder is equivalent to 50 mgms. of bacilli.

Inoculation of this product is often followed by the formation of painful abscesses. A. Fraenkel and Steffen however, claim to have had good results with it among their cases at Badenweiler. The treatment is begun with 0.001 mgms. and the dose is increased progressively up to 4 mgms.

XIX. Tuberculo-toxoidin of Ishigami. Ishigami[52] makes this preparation by treating bacilli previously dried and then washed, with sulphuric acid in concentrated solution. When diluted with 10 volumes of water the fatty and the waxy substances separate out upon the surface, and the insoluble precipitate, collected from the bottom by decanting, is emulsified in a weakly alkaline solution. The product is not toxic, even for tuberculous animals, although it is said to be still capable of provoking antibody formation and consequently of serving as an antigen.

XX. Tebesapin or Molliment No. 8 (the former Prosperol of Zeuner). In a communication before the Tuberculosis Congress at Washington in 1908, Noguchi called attention to the immunizing properties of tubercle bacilli macerated for 24 hours in *sodium oleinate* at 37°C. These experiments have not been confirmed; but at the same time Zeuner launched a preparation under the name of *Prosperol,* later of *Tebesapin,* and then finally under the name of *Molliment No. 8.* It is derived as follows:

Tubercle bacilli are macerated with continuous shaking for four days at 37°C. in a 2 per cent solution of sodium oleinate. They are next heated for one hour on a water bath at 70° to 72°C. and the shaking at 37°C. is continued for another three days. The emulsion is now concentrated to different strengths corresponding to a given number of milligrams of bacilli per cubic centimeter; No. 2 equals 0.5 mgms. of the *bovine* type per cubic centimeter; No. 5 to 2 mgms. of the *human* type; No. 6 to 10 mgms. of the *bovine* type; No. 9 to 10 mgms. of the *human* type, etc.

It is strongly hemolytic and for this reason cannot be utilized *in vitro* as an antigen for complement fixation.

[52] Philippine J. Sci., 1908, **3**, 379.

According to investigations by R. Moellers and Georg Wolff,[53] this preparation possesses no immunizing properties and exerts no favorable influence on experimental tuberculosis in the rabbit or guinea pig. Zeuner[54] claims that it can be advantageously given to patients by mouth or by rectum.

The clinical experiments carried out by Weicker on 50 patients at Görbersdorf are however nothing less than convincing. The commercial exploitation of this product is not justified on any scientific ground. Unfortunately the same is true of many similar preparations.

I shall limit myself to citing a few products which have been proposed by their originators for the treatment of tuberculosis without their having been tested experimentally. In the special articles concerning them will be found the indications for their administration.

Tuberculinum purum of Gabrilowitsch[55] (*Endotin,* albumin-free tuberculin).

Tuberculin of Maréchal (of Brussels). (A mixture of old tuberculin and guaiacol.)

Mycolysin-Tuberculin of E. Doyen. (A mixture of tuberculin and yeast juices.)

All of these products owe their properties to endo- and exotoxins contained in the original tuberculin of Koch or in purified precipitated tuberculin, and it does not appear that any of them replaces to advantage the original which is easily prepared and of perfect stability, provided it is protected from air and is in concentrated glycerin solution.

H. ACTION OF PHYSICAL AND CHEMICAL AGENTS ON TUBERCULINS

Dilutions of tuberculin are ordinarily made in 0.5 per cent carbolic water. They should be promptly used since they gradually lose their toxicity;—approximately one half in two weeks. It is therefore preferable, especially for laboratory experiments and even for therapeutic use, to make dilutions with physiological salt solution as need arises. Tuberculin diluted with plain water is very unstable and readily permits the development of microörganisms which modify or destroy its activity.

[53] Veröffentl. d. R. Koch. Stift. z. Bekämpf. d. Tuberk., 1913, H. 8/9. 74.

[54] Ztschr. f. Tuberk., 1909, **15**, 135; 1912, **19**, 268.

[55] Ztschr. f. Tuberk., 1908, **13**, 234; Beitr. z. klin. d. Tuberk., 1911, **19**, 485; **21**, 235.

Tuberculin in the crude concentrated state is not affected by light. Hans Jansen[56] found it still undamaged after two hours of exposure to the intense bright rays of a Finsen apparatus. Ultra-violet rays render it incapable of producing reaction in tuberculous guinea pigs only after five hours of exposure (A. Jousset, L. Massol,[57] M. and Mme. Victor Henri and Baroni).[58]

On the other hand, the digestive juices, trypsin in alkaline medium, papain in neutral medium, pepsin in acid medium and the digestive juices of insect-absorbing plants (Drosera), destroy it more or less rapidly whether *in vitro* or in the digestive tract (Carrière, Kinghorn,[59] Koehler, Th. Pfeiffer and Persch, Loeffler). Danielopolu[60] studied the action of hydrochloric acid and of pepsin separately through artificial digestion of precipitated tuberculin, at a temperature of 37°C. He was able to establish in this way that hydrochloric acid alone has no effect, that pepsin alone attenuates tuberculin quite strikingly and that mixtures of acid and pepsin destroy it after 24 hours.

The relative delay of digestion of tuberculin by hydrolyzing ferments permits of its partial absorption by the digestive mucosa, so that it produces its toxic effects when ingested in fairly large doses, especially in young animals whether healthy or tuberculous (Calmette and Breton),[61] or even in tuberculous human beings after alkalization of the stomach with bicarbonate of soda (Freymuth).[62] The toxic effect is still more marked if the tuberculin is ingested in keratin capsules which are dissolved only by the intestinal secretions, (Mollers and Heinemann)[63] (see Chapter XLII).

H. J. Bing and V. Ellermann[64] called attention to the fact that if an emulsion of egg-yolk lipoids (and more particularly of the ether-insoluble fraction, *albin*, which is a *diamidophosphatid*) be allowed to act upon tuberculin, the specific action of the latter is increased. The lipoids of caseous tissue (lymph nodes, liver, lungs) exert the same

[56] Centralbl. f. Bakt., 1906, **41**, 677; 775.

[57] Unpublished work.

[58] Compt. rend. Acad. des sci., 1910, **151**, 724.

[59] J. Med. Research, 1904, **12**, 213.

[60] Compt. rend. Soc. de biol., 1910, **68**, 896.

[61] Compt. rend. Acad. des sci., 1906, **142**, 616.

[62] München. med. Wchnschr., 1905, **52**, 62.

[63] Deutsch. med. Wchnschr., 1911, **37**, 1825.

[64] Biochem. Ztschr., 1912, **42**, 289.

activating effect, while cholesterol, oleic acid and its sodium soap produce no effect.

Danielopolu,[65] Moussu (of Alfort),[66] Zieler[67] and Haentjens have, on the other hand, shown that tuberculin, raw or precipitated and redissolved, passes through dialyzing bags of viscose, collodion or vegetable parchment; that the dialysis is slow and progresses best at a temperature of 37°C., and that the active substance likewise passes through porous candles introduced into the animal body.

[65] Compt. rend. Soc. de biol., 1909, **66**, 334.

[66] Ibid., 1906, **61**, 95.

[67] München. med. Wchnschr., 1908, **55**, 1685.

CHAPTER VI

HISTOGENESIS AND EVOLUTION OF THE TUBERCLE AND OF BACILLARY LESIONS WITHOUT TUBERCLES.

A. HISTOGENESIS AND EVOLUTION OF THE TUBERCLE.—ANATOMICAL PROCESS OF HEALING

Certain vegetable parasites (*Aspergillus, Actinomyces*), or animal parasites (*Nematodes, Acari, Sarcosporidia of the skin* of the ox) (Ch. Besnoit and V. Robin[1]), various bacteria (*Bacillus mallei, Bacillus leprae*), and also certain foreign bodies such as mercury, oil of turpentine, euphorbium powder, lycopodium powder or even coal dust impregnated with *Bacillus subtilis* (Marcel Garnier and A. Chaoul[2]) are capable of producing within the body tissues cellular reactions which terminate in the development of real tubercles. When however the latter are produced by substances other than living microörganisms they present the essential characteristic of not being inoculable in series. They are pseudo-tubercles.

The *true tubercle, produced by the tubercle bacillus*, appears first in the form of a *granulation*, a small, hard, barely elevated, grayish nodule, which cannot be enucleated and which is almost always surrounded by a reddish vascular zone. Its diameter varies from 0.1 to 3 mm. In the beginning, this small granulation is transparent and hyaline; then little by little it becomes more opaque at the center which takes on a yellowish tint. It then becomes the *tuberculous follicle* characterized histologically by a *giant cell* surrounded by a zone of *epithelioid* and *embryonal cells*. The outline of this small mass is irregular. Its size is that of the head of a pin or of a millet seed (miliary tubercle). There may be no further enlargement and the small mass may undergo fibrous degeneration ending in a small hard cartilaginous nodule containing atrophic elements, that is, healing. Several neighbouring tubercles may fuse and form a mass the center of which undergoes hyaline degeneration (Grancher) and caseation.

[1] Compt. rend. Soc. de biol., 1913, **75**, 442.
[2] Ibid., 1912, **72**, 1005.

The protoplasm, and next the nuclei of the giant cells become destroyed; so that nothing is to be distinguished except remnants among which the bacilli in relatively large number are irregularly scattered, especially at the periphery, inside the zone of epithelial cells. As the caseation continues the stainable bacilli diminish in number and end by apparently disappearing completely. Nevertheless a few of them always remain, since the caseous matter, when inoculated into a susceptible animal like the guinea pig, shows itself to be virulent and produces tuberculous infection.

In this stage of *caseous tubercle*, the lesion may still retrograde. In such case the embryonal cells surrounding the small mass become organized into fibrous tissue, forming a dense wall which thickens gradually up to the center, where ultimately only leucocytic debris is to be found in the sclerotic framework. A few granular malformed bacilli persist there for years in a dormant state (Metchnikoff), capable of being revived by experimental inoculation after the enveloping substance has been crushed, but ordinarily incapable of multiplying *in situ* in the lesion itself. In this manner the process of spontaneous healing of the tubercles is most often accomplished,—an apparent healing, rarely complete, since it is only exceptionally that some vestiges of caseous matter and a few bacilli do not persist indefinitely at the center. It does happen nevertheless in certain cases that the atrophy progresses to a point where nothing remains except a sort of cicatricial tissue which is hornlike in its hardness.

Under other circumstances, the sclerosed tubercles become infiltrated with calcareous deposits and transformed into veritable pearls which are hard and opaque and resist the knife like chalk. Inoculation with them, after grinding, proves that they still frequently contain bacilli which can be revived (L. Rabinowitsch, Piettre, Lubarsch).

After this short résumé of the different modes of evolution of the tubercle, the question arises as to the source of the cellular elements which compose it; a subject which has been the object of numerous works and is still in dispute. Until recent years, many histologists agreed with Baumgarten that the tubercle builds itself up at the expense of and through the proliferation of the fixed tissue elements. This opinion was defended by Kostenitsch and Wolkow,[3]

[3] Arch. de méd. expér.. 1892, **4**, 741.

by Klebs, Thoma, Stieck, Kockel, by I. Straus in his book published in 1895 on tuberculosis and its bacillus, and by Grancher.

Baumgarten[4] inoculated bits of tuberculous matter into the anterior eye chamber of the rabbit and was convinced that he saw the fixed cells of the iris divide by karyokinesis to form giant cells and epithelioid cells.

According to him, it is only secondarily that the leucocytes enter in and invade the small tumor formed by the fixed pre-existing cell elements produced by indirect division. In the lung a similar process is observed. During the first few days after infection with the bacillus, nothing is visible macroscopically although karyokinesis of the various fixed cells in the areas invaded by the bacilli can be seen microscopically. The bacilli become lodged upon the walls of the alveoli and bronchioles and fix themselves, in part upon the endothelial cells of the capillaries, in part in the interalveolar connective tissue. There they produce an irritation. The alveolar epithelium detaches itself from the basal membrane and accumulates in the center of the alveolus; the cell protoplasm becomes granular, while the endothelial cells of the vessels preserve their transparency.

In the connective tissue of the interlobular septa, in the walls of the vessels and bronchi and in the lymphatic follicles, there is always to be observed, according to Baumgarten, the same process of karyokinesis provoked by the presence of the bacilli.

A few days later the tubercles are visible to the naked eye. After still another few days histological study reveals voluminous tubercles formed from a cluster of alveoli packed with epithelioid cells. Then the tubercles become caseous and the further evolution shows nothing in particular. New tubercles continue to form and to unite themselves with the old, constituting the caseous masses. When the caseation becomes general, it is difficult to distinguish it from tuberculous lobar or lobular pneumonia, regardless of whether the latter conditions are produced spontaneously or artificially by inhalation of bacilli or by direct injection of tuberculous material into the lung.

"In this case," says Baumgarten, "infiltration of the lung is much more rapid. The tuberculous process is provoked immediately in a certain number of lobules; leucocytes appear earlier and in larger number. It is true that karyokinesis has not then been observed, but the identity of structure of the miliary tubercle and of that following

[4] Ztschr. f. klin. Med., 1885, **9**, 93; 245; — 1886, **10**, 24.

inhalation warrants the supposition that the same histogenic laws are applicable to both cases."

This theory of the formation of the tubercle *through proliferation of fixed tissue elements in reponse to irritation* has been actively and successfully opposed by Metchnikoff,[5] by Yersin[6] and the whole Pasteur school, especially by A. Borrel[7] to whom we are indebted for a most important experimental study of the pathological anatomy of the tuberculous process in the lung and in the kidney. By intravenous injection Borrel infected the rabbit kidney and was able to observe *in the absence of traumatism* the earliest expression of the infection, the immediate reaction of the animal body, a rapid formation of tuberculous granulations in the vessels themselves and their evolution through to caseation.

The study of the process of lung tuberculization led Borrel to observe the fact that the granulations, in this secondary period following infection, are always developed *in and from the lymphatic elements.* "In the lung as in the serous cavities, where Kiener first called attention to the fact, the site of election of tubercles about the vessels is due to the peculiarity that they develop almost exclusively *in the lymphatic system.* The latter is the matrix wherein the tubercles are formed, and not the connective tissue as claimed by Virchow. *The tuberculous cell is always a lymphatic cell* and is not derived in one instance from a lung cell, again from a liver cell, or still again from a kidney cell.

"These lymphatic granulations, so easily studied in the lung, constitute the true tubercles of the majority of investigators; they are the granulations of Laennec, the nodular tubercles of Virchow, the miliary granulations of Cruveilhier, the fibro-plastic granulations of Robin, Empis, etc.

"They exist in other organs exactly as in the lung. But in the latter because of its structure, the tuberculous matter may take the form of an infiltration, and we are led to the conclusion that *the tuberculous pneumonic process is not due to a desquamation of the epithelial cells of the alveoli* (as the partisans of the Baumgarten theory believed), *but to an exudation, into the interior of these alveoli,*

[5] Virchow's Arch., 1888, **113**, 63; Ann. de l'Inst. Pasteur, 1888, **2**, 505; *Leçons sur la pathologie comparée de l'inflammation*, Paris, 1892, Masson & Cie

[6] Ann. de l'Inst. Pasteur, 1888, **2**, 245.

[7] Ibid., 1893, **7**, 593; 1894, **8**, 65.

of lymphatic elements analogous to those which we find in the intralymphatic tubercles (A. Borrel)."

On killing a rabbit immediately after the intravenous injection of an even suspension of tubercle bacilli, Borrel finds first that almost all the bacilli are already contained within the polynuclear leucocytes disseminated more or less everywhere. By the end of 24 hours, however, leucocytes and bacilli are already localized. On the third day, the leucocytes containing bacilli are beginning to undergo degeneration, the nucleus in breaking up becomes more and more homogeneous and cloudy and the chromatin network and bodies are no longer visible. From the fifth day on there is no further trace of polynuclear leucocytes. Indeed by the second day, at the points where bacilli and polynuclear leucocytes are collected, one observes the influx of large mononuclear cells which are vesiculated, stain but poorly, and have an abundant protoplasm, giving off numerous projections. In view of the intravascular location their significance is not open to doubt, they are the large mononuclear leucocytes. Soon, from the third day, they can be observed grouping themselves about the masses of bacilli, fusing together and thus forming the typical giant cells (*see plate III*).

The number of nuclei contained in such protoplasmic masses can at times be very considerable, says Borrel, who was able to count as many as 60 of them. "Quite often, in a single plasmic mass the nuclei are arranged in groups and are almost always at the periphery, as a sort of collar. This marginal disposition of the nuclei is not difficult to understand when it is realized that in every mobile cell the portion deprived of its nucleus is the one which undergoes movement, while the cell portion containing the nucleus is always the relatively stationary part. In the presence of a small clump of bacilli, the mononuclear leucocytes situated on the vascular walls can be seen to send out extensions in the direction of the bacilli, the nucleus remaining always at the periphery. These pseudopods are at times very long and, from the progressive confluence of a large number of them, the giant cell results. In certain cases all of the nuclei are concentrated at one pole and the bacilli are situated in the non-nucleated portion of the cell. From the first days after inoculation such giant cells can be found forming in the alveoli by this same process."

PLATE III

Genesis of Giant Cells and Early Stage Tubercles
(According to A. Borrel)

1. Section of a large capillary of rabbit's lung in which are to be seen leucocytes, full of bacilli, and well isolated among the red cells (a few minutes after intravenous inoculation).

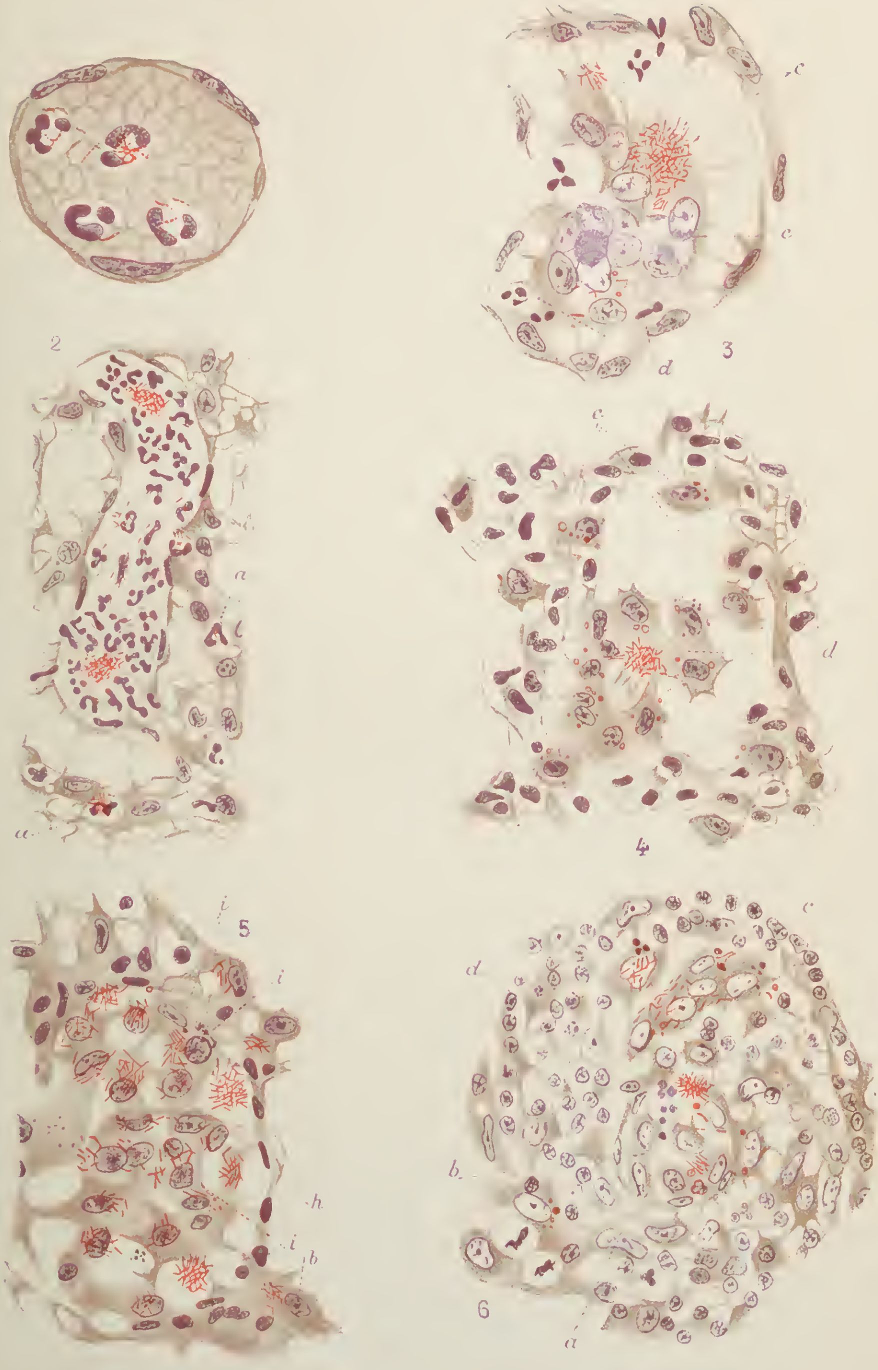
2. Longitudinal section of a dilated pulmonary capillary containing bacilli and numerous leucocytes which have already ingested bacilli a few minutes after inoculation; *a*, isolated leucocytes carrying bacilli.

3. A giant cell on the fourth day. The cell is in a capillary; the nuclei are concentrated at one pole; the bacilli are grouped at the opposite pole in the midst of a well defined felt-like mass of protoplasmic filaments; *d*, polynuclear leucocytes.

4. Formation of a giant cell from dust cells, in a lung alveolus. A mass of bacilli is in the center of the alveolus; all about are grouped a number of dust cells sending out protoplasmic extensions in the direction of the bacilli. On the alveolar walls there can be distinguished isolated dust cells, one of which is joining itself to the principal mass.

5. A mass of dust cells in the interior of the alveolus on the fifteenth day after inoculation. The bacilli have even developed in the interior of the cells; *b*, a bacillus-containing dust cell in a neighboring alveolus; *h*, wall of the alveolus; *i*, intra-alveolar cells.

6. Tuberculous granulation in process of formation on the third day. In the center one can distinguish the lumen of a capillary containing some mononuclear leucocytes and bacilli; all about the capillary, in the midst of the infltration of small round cells, large elements, which are either mononuclear leucocytes or dust cells. In *c* a giant cell with bacilli; *e*, a dust cell mitotic figure.


1
2
a
a
3
c
c
d
c
4
d
5
i
i
h
i
b
6
d
b
c
a
e

The phenomena are identical in all the organs when infected by way of the blood stream. They are likewise identical for all animal species naturally infected by way of the lymphatics, for example in cattle made to live with other tuberculous cattle and which are then slaughtered while infection is still localized in the glandular system (Calmette and Guérin).

In tuberculosis of the lung, Borrel has noted that the dust cells (*Staubzellen* of the Germans) play the same rôle in the interior of the alveoli as the large mononuclear leucocytes in the vessels. These dust cells, each made up of a very large vesiculated nucleus and dense granular protoplasm, are quite voluminous and have irregular outlines. They are adherent to the walls of the alveoli and are found spread out on the surface of the bronchial epithelium, moving in some way deep within the ciliated layer.

These cells, which have been carefully studied by Tchistowitsch[8] in Metchnikoff's laboratory, are certainly of lymphatic origin. They are contractile, ingest foreign bodies with the greatest ease, and form typical giant cells.

If a concentrated emulsion of *living or dead* bacilli is introduced directly into the trachea, or is inhaled (Calmette and V. Grysez), the alveoli, from the earliest days, are found to be invaded by an enormous number of dust cells. The majority of them contain bacilli and are as though drowned in an effusion of polynuclear leucocytes which soon undergo processes of degeneration. On the fourth or fifth day of the acute pneumonia, the alveolar cells are filled with chromatic granules. The walls of the alveoli are intact, the epithelium is in place, and yet the alveoli are filled to overflowing. Multiplication of the epithelial cells by karyokinesis cannot be invoked to explain this invasion (Borrel).

If one examines the *liver* of an animal experimentally infected, it is seen, as Metchnikoff has shown, that *the tubercle cells, both epithelioid and giant cells, are formed exclusively from the large mononuclear leucocytes and from the* Kupfer *star cells, which are of endothelial origin.* Neither the hepatic nor epithelial cells contribute to the formation of the tubercle. It is true that at times a few such elements are found in process of karyokinetic division, but this proliferation has no direct relationship with the formation of the tubercle and serves only to regenerate the specific elements of the hepatic tissue.

[8] Ann. de l'Inst. Pasteur, 1889. **3**, 337.

PLATE IV

Phagocytosis and Modifications Undergone by the Tubercle Bacillus Within the Giant Cells of the Gerbille

According to Metchnikoff

1. Giant cell from the spleen of the *gerbille*. *a*, capsule of the bacillus; *b*, Bacillus of Koch. The spleen, fixed with Flemming's solution, has been stained by Gram and with eosin. Oc. 3; imm. obj. $\frac{1}{18}$ Zeiss.

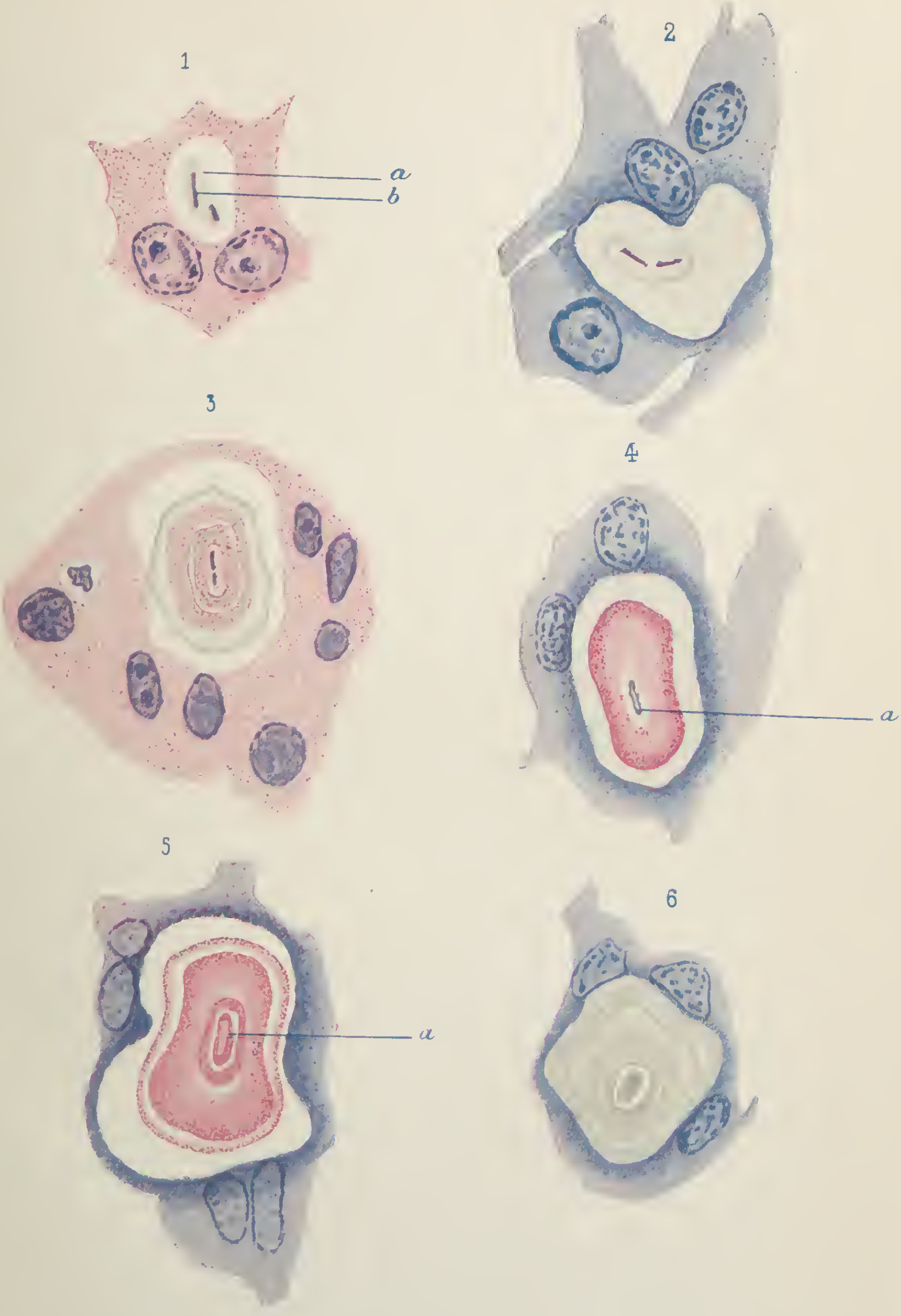
2. Giant cell from the spleen of the *gerbille*, enclosing a calcareous body with a double bacillus. Same magnification. Stained with hematoxylin and Ziehl fuchsin.

3. Another giant cell from the spleen of the *gerbille*, enclosing a bacillus surrounded by concentric layers. Same staining. Oc. 2; obj. $\frac{1}{18}$ Zeiss.

4. Giant cell with a calcareous body which contains only a trace of the bacillus *b*. Fuchsin-hematoxylin; same magnification.

5. Another giant cell in which the bacillus *a* has been transformed into a body stained light pink.

6. A giant cell containing a calcareous body definitely formed.


1
a
b
2
3
4
a
5
a
6

Tubercles of the spleen and those of lymphatic glands develop in like manner following an accumulation of the large phagocytes of these organs, so that the process of giant cell formation is always the same.

Bowman, Winternitz and Evans[9] demonstrated that it is possible to vitally stain giant cells and tubercles in process of formation, in the rabbit, by means of intravenous injections of about 20 cc. of a 1 per cent solution of trypan blue, provided the injections are made not more than a half hour after the intravenous injection of the infecting bacilli. The tuberculous cells stain an intense blue and appear sharply differentiated upon the vascular walls, especially in hepatic tissue.

Metchnikoff noted a very characteristic degeneration of human and avian tubercle bacilli in the epithelioid cells and especially in the giant cells of *spermophiles*, animals which, in general, are quite resistant to tuberculosis. The bacilli became swollen and gradually lost their capacity to retain anilin dyes. "Most often it is the central part which first decolorizes; at times it is the peripheral portion. Later the bacillus becomes transformed into a yellowish sausage-shaped body in the interior of which a very narrow canal is to be seen. The bacilli, thus malformed, unite to make up a mass which takes on the characteristic appearance of a piece of amber. At this time they are conspicuous because of their brownish staining. All of these transformations are never observed, either in cultures (even though many dead bacilli are present), or outside of tuberculous cells."

Similar transformations of bacilli have also been demonstrated in the giant cells of rabbits and, very rarely, in those of guinea pigs. They are not found in cattle or in man, although in these species bodily resistance is often very marked.

"For along time," says Metchnikoff,[10] "calcification of tubercles as a mode of healing of tuberculosis in man, has been observed. In order to give a more exact idea of this reaction phenomenon I may cite the case of the resistance to the tubercle bacillus on the part of the body of the *gerbille*[11] of Algeria (*Meriones shawii*). This rodent, which

[9] Centralbl. f. Bakt., 1912, **65**, 403; J. Exp. Med., 1914, **19**, 283.

[10] *Leçons sur la pathologie comparée de l'inflammation.* Paris, 1892, Masson & Cie.

[11] Gerbille or Gerbil, the name of a group of small jumping rodents forming a special subfamily of the rat tribe or Muridae.

is not absolutely refractory to tuberculosis, resists infection much more effectively than most animals of the same species. *Meriones* inoculated subcutaneously, and even into the eye, with a culture of human bacillus, resist infection for several months.

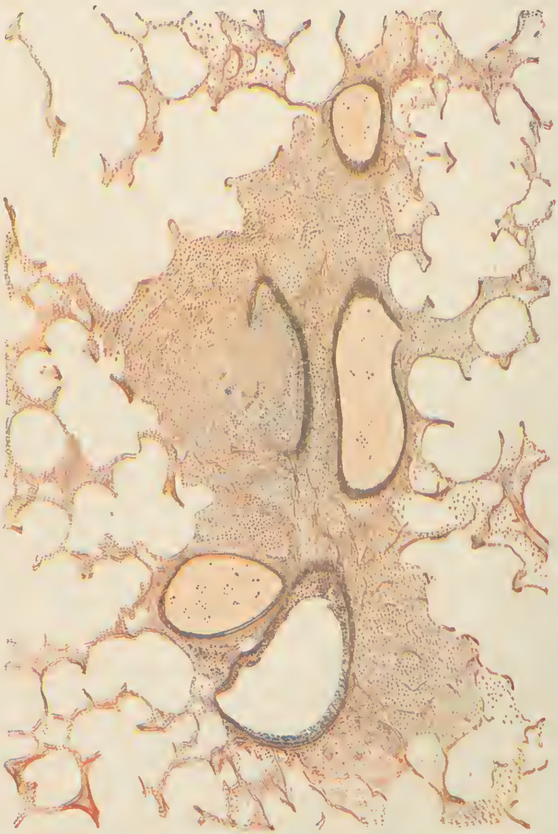


FIG. 6. INVASION OF THE AIR PASSAGES IN MILIARY TUBERCULOSIS

Wall of intra-lobular bronchiole being broken through (from a preparation of Professor Letulle).

"If the *Meriones* are killed 6 to 8 months after inoculation, one finds a large number of tubercles in the abdominal organs, in the lungs and in the lymph nodes. Such tubercles however, in the majority of cases, present none of the phenomena of necrosis and caseation.

"The tuberculous tissue, made up of vigorous live cells, contains bacilli of which the great majority show a very remarkable degeneration meriting more detailed description (*see plate IV*).

"The spleen in particular is sown with small tubercles composed of epithelioid and non-necrosed giant cells. The tubercle cells enclose a small number of ordinary tubercle bacilli, while the giant cells contain very characteristic calcareous bodies. On examination under the microscope, these bodies for the most part have the shape of the figure 8, and are very refractile. At times their form is simply round or irregular. Under the influence of acids, the calcareous salt (calcium phosphate) is dissolved, leaving a more or less numerous series of concentric fairly thin layers.

"The calcareous bodies bear great resemblance to the formations described by Schueppel[12] in scrofulous lymph glands and found by several authors in many cases of glandular tuberculosis in man (Ziegler). But while, in the latter, the origin of the striated calcareous bodies is still completely obscure, in the gerbille it can be easily demonstrated. Examination of the preparations spread on slides, or of sections stained by Ziehl, shows at once that these calcareous bodies reveal a state of degeneration of the tubercle bacilli in the interior of the giant cells. In the early stages the bacilli stain normally, but there are found other giant cells in which the bacilli are encapsulated with a fairly thick layer of an amorphous unstained substance.

"This secretion becomes more and more abundant, so that the bacilli are found enclosed within several concentric layers. Sometimes in the center of a calcareous body a bacillus is seen divided into two parts, one half stainable, the other half no longer so. Through a series of intermediate changes decolorized bacilli are ultimately found, traces of them being still present in contour. But eventually the bacilli are no longer to be distinguished from the surrounding tissue and in the final stages they disappear completely. This last stage, which is by far the most frequent, is that of the stratified calcareous bodies. . . . .

"The struggle of the two living organisms—the tubercle bacillus and the giant cell of the gerbille—is carried on therefore with the help of secretions. The bacillus defends itself by the secretion of the

[12] *Untersuchungen über Lymphdrüsen-Tuberkulose sowie über die damit verwandten und verwechselten Drüsenkrankheiten.* Tubingen, 1871, Laupp.

cuticular membranes and probably also by the production of toxins, while the giant cell secretes a calcareous deposit by the aid of which it walls in the bacillus and finally destroys it in a very large number of cases."

B. PROCESS OF CASEATION OF TUBERCLES

The mechanism of *caseation of tubercles* is not yet fully understood. This caseation, in the opinion of certain workers, is associated with a deficient blood supply, the tubercles being deprived of vessels. According to others, it results from the action of the toxins produced by the bacillus.

Auclair[13] believes that the specific poisons are of the nature of fats and are soluble in ether, chloroform, benzin and xylol. The extracts from these different solvents, emulsified in water and injected into the subcutaneous tissues, cause the formation of cheesy abscesses. When injected into the trachea of the guinea pig, caseous areas appear.

Ethero-bacillin in particular (the preparation of which was described in Chapter IV) seems to be the bacillary poison *which causes caseation,* while the *chloroformo-bacillin* appears to be the *toxin which induces sclerosis.*

However it is known today that the caseating property attributed by Auclair to the fats of the tubercle bacillus which are extractable with ether, is in no wise specific. The fats extracted by the same method from many other bacteria, such as paratubercle bacilli (timothy bacillus, bacilli from dung, maize, etc.), diphtheria bacilli and the *Bacillus subtilis* itself, are capable of producing absolutely similar necrotizing and caseating effects when introduced into animal tissues.

Joest[14] found that in miliary tubercles there is no fat, while in older tubercles it is found in the very center of the focus, and fatty degeneration precedes the necrotic changes. In very old lesions fatty degeneration is found only in the zone between the dead (necrosed) and living tissues.

The deposit of fat is intra-cellular and is in the giant and epithelioid cells. It is not produced either in the leucocytes or in the intercellular spaces. Excess of fat is the prelude to cell death and its origin lies in the toxic products of the bacillus. The effect of the toxic

[13] Thése, Paris, 1897; Arch. de méd. expér., 1899, **11**, 363; 1900, **12**, 189.
[14] Ztschr. f. Infektionskr. . . . d. Haustiere, 1911, **10**, 120.

products depends on their concentration for if present in but small amount they cause a cellular proliferation; if present in large amount, they cause a fat excess and then a necrosis.

The investigations of H. Dominici and Ostrovski,[15] with regard to the action of the diffusible toxins of the tubercle bacillus upon normal

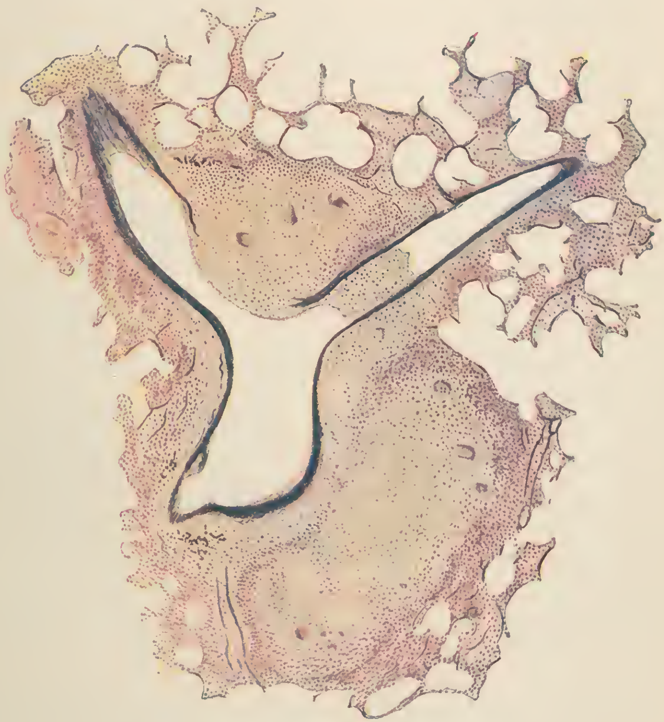


Fig. 7. Lesions of the Pulmonary Vein

Tuberculous nodules and conglomerate miliary granulation.

Tuberculous peri-phlebitis; a tuberculous focus breaking through a vein wall. Lung of a child (from a preparation of Professor Letulle).

tissues, brought proof that the lesions most characteristic of tuberculosis (tuberculosis undergoing sclerotic or caseous evolution, atrophic sclerosis, dry or liquefying necrosis, caseation) can be produced, *remote from the point of inoculation*, by injecting into the

[15] Compt. rend. Acad. des sci., 1913, **157**, 1171.

cellular tissue of a guinea pig, a fluid obtained by macerating (in distilled water at 42°) living bacilli treated with pure sulphuric ether and then washed to remove all traces of the broth culture. This liquid, filtered through a Chamberland bougie, is an aqueous solution of protein substances, some of them dialyzable and others in a colloidal state.

In other experiments, the same workers first treated the bacilli with ether and then macerated them at 70°C. in distilled water. The liquid, filtered through a Chamberland filter and concentrated to one-tenth of its original volume, was subjected to dialysis in order that the dialyzable substances and the colloidal substances might be utilized separately. The injection of this extract was followed by reaction phenomena of cellular hyperplasia, localized particularly in the lymphoid organs and in the lung. With the dialyzable substances there were observed in addition very small disseminated lesions of necrosis, which were lacking when the colloidal substances were employed alone.

It is therefore impossible to attribute the origin of the process of caseation to the fats and to the waxes which make up the larger part of the ectoplasm of the tubercle bacillus. It is much more probable that the cellular ferments play the principal rôle.

In caseous pneumonia, for example, many cells die and disintegrate when the inflammatory exudate, originally composed of dust cells, leucocytes and fibrin, becomes rapidly caseous. Now these cells contain ferments which, according to Jobling and Petersen,[16] are inactive because the tubercle bacillus itself contains a substance, probably *a lipoid soap*, which is *inhibitory*.

It is known in fact that trypsin becomes inactive when exposed to a temperature of 30°C. in the presence of non-saturated fatty acid soaps, and Jobling and Petersen have demonstrated that the bodies of the tubercle bacilli contain non-saturated fatty acids which, when saponified, inhibit *in vitro* the action of trypsin and leucocytic protease.

The absence of autolysis in caseation, as in anaemic infarct, is said to result from substances of the same nature.

It should be granted then that *caseation of tubercles is the result of the direct and localized toxic action of the bacilli and of their diastatic or toxic secretions upon the giant cells which contain them.* It shows

[16] J. Exper. Med., 1914, **19**, 251; 383.

itself histologically in *a granulo-fatty degeneration* and a *nuclear fragmentation* (Chaussée and L. Pissot).[17]

The center of the giant cell becomes a culture medium for the development of a greater or lesser quantity of bacilli. When, with the progressive softening of the caseous material, a perivascular tubercle breaks into a blood vessel or into a neighboring lymphatic space, the enclosed bacilli, swept into the lymphatics or the blood stream, become quickly the prey of new polynuclear leucocytes and then of

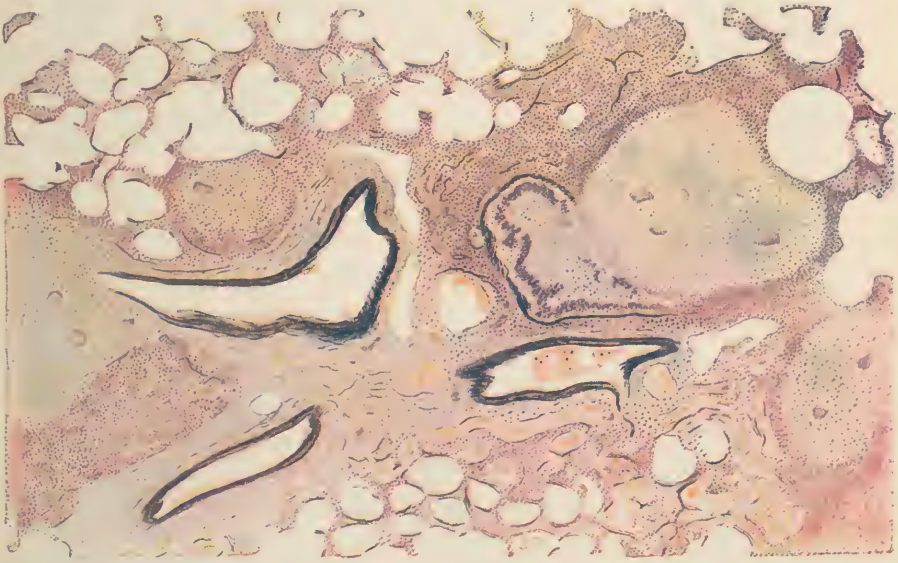


FIG. 8. NODULAR TUBERCULOSIS

Destruction of a bronchiolar wall by a caseous tuberculous nodule (from a preparation of Professor Letulle).

mononuclear cells which will reproduce other and similar tubercles either near or far from the point of initial infection.

If the caseous tubercle ruptures into a lung alveolus or into a small bronchus, it produces at first an isolated area of tuberculous pneumonia, then of tubercle formation in the alveoli, for which the fixed cells of the lung serve as a passive support until they become dissoci-

[17] Compt. rend. Acad. des sci., 1911, **152**, 108.

ated and destroyed in their turn by the progressive extension of the caseous process.

"One may state," says Letulle, "that aside from the examples of primary tuberculous pneumonia of purely respiratory origin, which are in truth very rare, and experimental so to speak, there are innumerable cases in which the vascular origin of pneumonic caseous lesions cannot be questioned.

"As an example, *miliary tuberculosis* of the lungs (the hematogenous or lymphogenous origin of which according to the case, cannot be doubted) by breaking through a neighboring wall, gives rise often prematurely to the complete series of specific obliterating changes in the bronchioles, which are the most important causal elements of caseous pneumonia." This is shown very well in figures 6, 7 and 8.

The tubercle therefore is truly a lymphatic production in all cases. The fixed cells of the different organs where it develops play no active rôle in its histogenesis.

C. TUBERCLE BACILLUS LESIONS WITHOUT TUBERCLE FORMATION

Until the last few years it was accepted that in all forms of tuberculosis described by clinicians and pathologists the presence of tubercles was an essential characteristic (miliary tuberculosis, caseous pneumonia, scrofula, etc.). Contemporary studies however show that infection by the bacillus may exist, and may be produced experimentally in animals, without any tubercles being formed.

Landouzy[18] and his pupils, Léon Bernard, Salomon, Gougerot,[19] Laroche, Jean Troisier, must receive the credit for having, since 1882, drawn and held the attention of observers to the previously unrecognized forms of tuberculous infection without tubercle formation (*non-follicular bacillosis*) which, as we know today, may involve all tissues and all organs (*see Chapter VII*, B).

Proof of this was brought by those whom I have just named, as well as by Darier, André Jousset and Braillon, Claude, Oettinger, Triboulet, and the work of Poncet and his pupils, although the latter have gone much too far in asserting, without histological or experimental proofs, that "the various disease accidents ocurring in tuberculous patients should in general be regarded as manifestations of the tuberculosis."

[18] Leçons cliniques de La Charité, 1881–86; de l'Hopital Laennec 1891–1910.
[19] Thèse, Paris, 1908.

Léon Bernard first, and later A. Jousset and Gougerot adduced a number of definite facts to prove the existence of these *inflammatory reactions without tubercles* which are capable of being produced through tubercle bacillus infection. Anatomically they have been found in the kidney, in the skin, in the endocardium, etc., and no one longer denies that it is possible for them, through an extension to "fluid tissue" such as the *blood*, to establish a more or less limited *septicemia* which frequently precedes the primary localization and almost always the secondary localizations (*see Chapter XVIII*).

L. Rénon and E. Géraudel[20] studied the *non-follicular bacilloses* from the histological point of view. They clearly brought out the fact that the *granulation*,[21] the *tubercle*, the *nodule*, are macroscopic forms but should not be regarded as histological realities. In the different organs these "nodular" lesions are lesions of *inflammation* and, in the lung, they are lesions of *pneumonia*, borrowing their special appearance from their limited extent and their focal arrangement.

"The composite anatomical picture found in a tuberculous lung," says Rénon, "corresponds to the aggregate of the successive pneumonic extensions which make up the clinical history of every pulmonary tuberculosis. To these pneumonic lesions tubercle formation may or may not add itself here and there; but the essential element of the lesion is always the *pneumonia*."

This conception is obviously the only one which enables us to understand why the tuberculous infection, according as it is *single* or *repeated*, according as it is *caused by bacilli of greater or lesser virulence and in greater or lesser number, in individuals tubercle-free or relatively immunized by one or more previous benign infections*, manifests itself at times by *small localized foci of inflammation* which tend to fibrous transformation and healing; again by *foci of severe inflammation* resulting promptly in cell *necrosis*, and afterward in the formation of true *tubercles*.

[20] Compt. rend. Soc. de biol., 1913, **75**, 699.
[21] See Chap. VI, §A, for explanation of these terms.

CHAPTER VII

THE PRINCIPAL PATHOLOGICO-ANATOMICAL TYPES OF INFECTION BY THE TUBERCLE BACILLUS

A. TUBERCLE BACILLUS SEPTICEMIA.—MILIARY TUBERCULOSIS

Infection of the human body by the tubercle bacillus is not always manifested from the outset by the formation of tubercles. It frequently happens, especially in the young, that the infection assumes the guise of a general infectious disease *similar to typhoid fever*, without any localized lesion to cause the formation of giant cells and the later evolution of the tuberculous process, and without any characteristic incubation period.

The clinical entity of this form of tubercle bacillus septicemia was established in 1882 by Landouzy,[1] who called it "*typho-bacillose*" by reason of the frequent resemblance of its symptoms to those of true typhoid fever, from which it is differentiated by the absence of intestinal catarrh and rose spots, and by the frequent presence of tubercle bacilli in the circulating blood.

When benign and atypical it frequently causes errors of diagnosis, being mistaken for a protracted mild form of typhoid fever or for a manifestation of grippe.

When quickly fatal, which is exceptional, there is no ulceration of Peyer's patches nor any visceral localization, only the diffuse congestive lesions in the different viscera, as in all septicemias, and at times a few small gray translucent granulations "not enough to cause local symptoms and only just sufficient to establish the identity of the disease."

"In the great majority of cases," states Landouzy, "after 3 to 4 weeks of continuous fever, accompanied by more or less marked prostration progressing generally to a definite typhoid state, with dry tongue and more or less hypertrophy of the spleen (a condition which,

[1] J. de méd. et de chir. prat., 1885, 488;—Leçons cliniques de La Charité et de l'Hopital Laennec 1885–1891; Semaine méd., 1891, **11**, 225;—Presse Méd., 1908, **2**, 681.

according to the intensity of the manifestations, is diagnosed as typhoid fever, a mild typhoid, or a febrile gastric disturbance), the patient begins to convalesce.

"Usually however the convalescence is not genuine; the patient does not regain his normal spirits; the keen appetite of the typhoid convalescent fails to appear; the lost weight is not regained. After a few weeks or months, there appear abruptly or stealthily the signs of a localization of the tuberculosis, most frequently pulmonary or pleural; rather often meningeal in the child.

"Let us take for example the case of a boy of 7 years, without past history of illness, suddenly taken ill with fever absolutely like a typhoid of moderate intensity, except for the absence of intestinal catarrh and rose spots. At the fourth week the child begins to convalesce and is taken to the country; he returns looking well but his cheeks are less full and he is less active than is ordinarily the case with a child after typhoid fever. The winter passes without trouble; then one morning, he does not feel well, has a headache, vomits, has some fever, and convulsions. In a few days death occurs from tuberculous meningitis.

"At times the convalescence which follows the "typho-bacillose" appears entirely frank and genuine; apyrexia is complete, cure is all but attained; and yet here again a localized tuberculosis intervenes sooner or later, and more or less abruptly.

"These cases which simulate typhoid infection (*typhoiques bacillaires*), although cured of their fever, almost always remain in a state of "gestation" as regards their tuberculosis; and a few weeks, a few months or several years after the initial acute septicemia they reveal themselves tuberculous. They develop the pathology and symptomatology of tuberculosis therefore only after having passed through the stage of bacillosis." (Landouzy.)

Acute tubercle bacillus septicemia was produced experimentally by Yersin in 1888, later by Gougerot in the rabbit and by C. Guérin and myself in 1906 in the bullock. In these animals, as in man, it terminates at times in complete cure without the formation of visible tubercles in any organ. However, except when the animals have been infected with bacilli culturally attenuated or modified in virulence, tuberculous localizations are always observed in the various lymph gland groups and in the lungs. The disease then develops in its usual chronic form, or remains in the state of *latent* tuberculosis.

Present-day clinicians, and particularly the pediatricians, recognize that tubercle bacillus septicemia is extremely frequent, either as a primary manifestation or (probably much more commonly) secondary to a sudden discharge into the circulation from a primary and up to that time latent tuberculous focus (tracheo-bronchial, for example) (Hutinel). Until described by Landouzy, Gougerot, Loederick, Widal, Hutinel, Weil and Mouriquand, Léon Bernard, and others, it passed unnoticed. Undoubtedly it plays a considerable rôle in the pathogenesis of tuberculosis and is probably very often an important factor in immunization. We shall have occasion to return to this subject.

In addition to this last type of infection there occurs fortunately much less frequently, the *massive* tuberculous infection, apparently primary, which manifests itself in man by a subacute septicemic form with more or less immediate single or multiple localizations. Again to Landouzy and his pupils are we indebted for the best study. The clinical type may first evolve like the preceding and end with a pleurisy, a meningitis or a more or less "galloping" phthisis; or else it may lead from the beginning to an acute miliary tuberculosis, the "granulie" of Empis, characterized by the early formation of an immense number of small gray translucent tubercles with caseous opaque centers in most of the viscera, especially in the lungs.

In the course of this severe form of infection there is frequently observed, particularly in young children, a specific cutaneous eruption which takes the form of disseminated and discrete papules scarcely larger than the head of a pin. These papules are soon transformed into vesicles which break and dry, leaving a little crust surrounded by a zone of brownish pigmentation. The eruption occurs in several successive outbreaks over a number of days and weeks, on the buttocks, the genital organs, the inner surfaces of the thighs, the abdomen, and more rarely on the chest. It results from the septicemia and the accumulation of tubercle bacilli in the skin capillaries. Tileston[2] was able, in 70 per cent of the many cases which he observed, to demonstrate the presence of the bacillus in the papules, as did Landouzy and Gougerot in a case of erythema nodosum (*see Chapter XVII*).

Acute miliary tuberculosis ("granulie") results from the irruption into the circulatory system of a large quantity of tubercle bacilli having their source in the softened caseous matter of one or more

[2] Arch. Internal Med., 1909, **4,** 21.

tubercles constituting the true primary localization. An intense febrile reaction is produced, the temperature rises and remains in the neighborhood of 39.5° to 40° C. until the end of the illness, which almost always terminates in death in 2 to 8 weeks, depending upon the severity of the infection and whether the miliary tubercles are particularly abundant in the nervous centers or in the lungs. In some rare instances where the miliary eruption was less intense, the acute symptoms have been seen to improve and the disease to develop into a chronic tuberculization. Such an outcome is however, quite exceptional.

B. LATENT TUBERCULOUS INFECTION

Infection by the tubercle bacillus manifests itself most commonly, in man as well as in naturally susceptible animals, by the slow formation of tubercles in one or more groups of lymph nodes more or less distant from the site where the virus originally penetrated the body. It was formerly believed and is still frequently taught, altogether wrongly, that the infecting element always leaves its mark at the point where it enters. The author of this doctrine, Cohnheim,[3] has formulated it into a law in the following terms:

At whatever point the tuberculous virus is introduced and remains during a sufficient period of time, a tuberculous or scrofulous lesion is created. As regards the primary localization, the portal of entry of the virus plays the important rôle in the primary localization. Once introduced into the body the virus propagates itself and is disseminated in accordance with local conditions, following the natural lymphatic and venous channels.

In the chapters to follow, in studying the mechanism of tuberculous infection, we shall see that the infection first occurs unobtrusively, and that unless it is effected in massive form (as happens at times accidentally, or as is almost always the case in experimental inoculation of animals), it remains latent in the lymph or blood system for a longer or shorter time and discloses its existence only by conferring upon the infected organism the capacity to react to tuberculin. *It is only after this varyingly prolonged period of latent blood or lymphatic infection that a primary tuberculous lesion is created, with or without the formation of tubercles, and the localization or fixation* of the primary lesions is determined either by certain anatomical relationships

[3] *Die Tuberkulose vom Standpunkte der Infectionslehre*; Leipz., 1880; 2nd edition, 1881, French translation by Musgrave-Clay, 1882.

within the organs (such is the case with the lungs), or by other circumstances, mechanical, physiological or accidental, which bring about the arrest, against the endothelial wall of a lymphatic or blood capillary, of a polynuclear leucocyte containing one or more phagocyted bacilli.

At the present time it is well established *that apparently normal lymphatic glands, removed at autopsy from subjects who have died at all ages whether from acute diseases or following accidents, harbor living and virulent tubercle bacilli whose presence is revealed only by the inoculation of these glands into very susceptible animals, such as the guinea pig.* As early as 1890–92, Loomis,[4] then Pizzini[5] had brought proof of this, and the proportion of *latent bacillus carriers* found by them in the course of their autopsies of *non-tuberculous* subjects was, for Loomis, 26.6 per cent; for Pizzini 42 per cent.

Proofs of a similar nature were later furnished by Spengler,[6] Kälble,[7] A. MacFadyen and MacConkey,[8] Harbitz, Weichselbaum and Bartel,[9] Rosenberger.[10]

With C. Guérin and Deléarde[11] I have myself demonstrated the frequency of latent infection of the tracheo-bronchial nodes, *without evidence of pulmonary lesions,* in infants dying from non-tuberculous diseases and presenting no clinical signs of tuberculosis. These facts have since been experimentally confirmed by several observers (Goodale, H. Wright and Smith, L. Rabinowitsch,[12] Lignières, Moussu, Vallée, Junack). Particularly abundant proof has been furnished by the veterinarians who, in the abattoirs, have systematically inoculated guinea pigs with the lymphatic glands of calves or swine which showed no evidence of any tuberculous lesion (Joest, Noack and Liebrecht,[13] Rievel[14] Linnenbrink, Jonske, Nieberle,[15] Emshoff and Semmler, Haeutle,[16] and others).

[4] Studies from the Loomis Lab., 1890, Vol. 1.
[5] Ztschr. f. klin. Med., 1892, **21**, 329.
[6] Ztschr. f. Hyg., 1893, **13**, 347.
[7] München. med. Wchnschr., 1899, **46**, 622.
[8] Brit. Med. J., 1903, ii, 129.
[9] Wien. klin. Wchnschr., 1905, **18**, 241.
[10] Am. J. Med. Sci., 1905, **130**, 95.
[11] Compt. rend. Acad. des sci., 1906, **142**, 1136.
[12] Berl. klin. Wchnschr., 1907, **44**, 35.
[13] Ztschr. f. Infektionskr. . . . d. Haustiere, 1907, **3**, 257.
[14] Deutsch. tierarztl. Wchnschr., 1909, **17**, 347.
[15] Ztschr. f. Infektionskr. . . . d. Haustiere, 1913, **13**, 59; 141.
[16] Centralbl. f. Bakt., 1914, **74**, 91.

The discrete glandular infections, that is to say those which result from a small amount of virus and are produced by attenuated bacilli, tend usually to remain *latent*. They then play, as will be seen later, a very important rôle in immunity against tuberculosis. They are very frequently encountered at autopsy, especially in children and adolescents, occurring most commonly in the peribronchial and mediastinal nodes, then, in diminishing order, in the glands of the neck, of the axilla, of the groin, in the cubital and popliteal, and finally in the retro-auricular and submaxillary glands.

In the adult, after the twentieth year, they are found much more rarely or, to be more exact, are less apparent; as the glands are then the seat of sclerotic lesions which cannot always be discovered on macroscopic examination and which are visible only under the microscope. In the majority of cases these sclerotic lesions still contain bacilli, at times very few and impossible to disclose in sections, but alive and virulent as shown by animal inoculation.

C. PROGRESSIVE TUBERCULOUS INFECTION.—PREDOMINANCE OF PULMONARY LOCALIZATIONS

It is a well known fact that *in tuberculous infection*, in *man as well as in cattle, pulmonary localizations are the most frequent*. This does not imply that they are the *first* from the standpoint of priority, for in reality they are almost always of *later date and consequent to tubercle formation in a gland of the cervical, tracheo-bronchial or mediastinal groups*. But it is the lung localizations which, in the majority of cases, make known their presence by the more or less serious functional disturbances (cough, hemoptysis, attacks of congestion) which characterize the early stages of the disease.

This *predominance* results from the fact that in the loose connective tissue surrounding the alveoli and the small bronchi, the lymph spaces and blood capillaries are the seat of a circulation which is less rapid than in any other organ, and this slowing is particularly marked at the *apices, which have a lesser degree of elasticity as well as smaller blood vessels*. Dust in suspension in lymph or blood, bacteria and degenerated or dead leucocytes have, as a result, a natural tendency to be retained there. *Adherence to the walls of the lymph or blood capillaries occurs here with more intensity than elsewhere*. The anterior margins of the middle lobes and the circumferences of the lower lobes possess the same character in lesser degree: there too the

localizations of tuberculosis are often observed and these same parts are the most frequent site of parenchymatous inflammations of bacterial origin (pneumonia in man, glanders in the horse, parapneumonia in the ox, and various infectious diseases in a large number of animals).

At times it happens that the tubercles are but few and are scattered among several lobules; again they form real conglomerate masses round about or in the center of the same lobule or of the same lobe; or again they appear in small isolated groups which tend to coalesce through the large number of smaller younger tubercles encircling the older larger lesions. Caseation then involves a part of a lobe or even an entire lobe, producing large cavities, being surrounded at times by an inflammatory zone of broncho-pneumonia and hepatization, which is red or gray according to the amount of infiltrating caseous matter or the degree of secondary bacterial infection.

All of these lesions may combine to present an infinite variety of aspects. They encroach little by little upon the surrounding tissues, following always the lymphatic channels. Grancher [17] insists quite rightly on this fact which he had carefully noted:

"*The blood vessels,*" says he, "*and specially the lymphatic vessels, are the true paths of conveyance of the miliary tubercle.* In fact, when tuberculous granulations are discrete, they can very easily be seen sowing themselves one by one in the interlobular spaces and thus circumscribing the base of the lobules. Often, where the spaces are very large, three or four tubercles join together and form a sort of small focus, whence they radiate out always along the lymphatic route. The tuberculous matter behaves like an injection fluid; it follows the path of least resistance and there are often found, at a fairly great distance from the focus of infection, tuberculous granulations which occupy the perilobular lymphatics long before the lobule itself is involved."

The caseous softening of the tubercles, the inflammatory infiltration of the neighboring tissues, the degeneration, the destruction, the defensive reactions of which these tissues are the seat, remain limited to the lungs but rarely. They frequently extend to other organs.

[17] Arch. de physiol. normale et path., 1878, 2. s., **5**, 1.

D. LOCALIZATIONS IN THE PLEURA

One would expect the pleura, whose lymphatic system is in direct communication with that of the lung, to be very frequently involved, and this is indeed the case.

In those affected with phthisis the walls of the serous sac are almost never free from the disease. Adhesions or serous effusions more or less rich in leucocytes and bacilli develop (dry pleurisy or pleurisy with effusion). It is now known, through the work of Landouzy, Kelsch and Vaillard,[18] Netter, Weichselbaum, Gombault and Chauffard,[19] Le Damany,[20] Prinz Ludwig, and others that pleurisies are almost always of tuberculous origin. "Every individual," says Landouzy, "who cannot furnish a satisfactory reason for his effusion, whether an infection (scarlet fever, puerperal fever, etc.), a dyscrasia (rheumatism), or a trauma (fracture of the rib, pulmonary infarct) is a tuberculous subject, even though he is plump and well nourished, and declares that he is well and that there is no past history of tuberculosis in himself or in his family. So-called pleurisy *a frigore* is ordinarily a manifestation or vicarious expression of tuberculosis."

But tubercles of the pleura, when not concomitant with wide spread lesions of the lung, tend generally to become sclerotic. They represent a benign form of tubercle bacillus infection which, at least to outward appearances, is usually capable of cure (*see Chapter XIII*).

E. OTHER LOCALIZATIONS

Along with its localization in the lungs or pleura or at an isolated point, the tuberculosis may, from the beginning, localize or sow itself in any organ provided with a plexus of lymphatic or blood vessels; but it has an evident predilection for serous membranes both visceral and articular, for lymphatic glands both superficial and deep, and for mucous membranes and skin where lymphatics are most abundant (phlyctenular conjunctivitis for example). It never *primarily* invades the highly specialized tissue elements. The latter are involved only *secondarily*, through extension of the processes of caseation or sclerosis which, as we have already seen, complete the evolution of the tubercle.

Whether we have to do with lupus, with tubercles in different parts

[18] J. de physiol. normale et pathol., 1906, **8**, 162.
[19] Semaine Méd., 1896, **16**, 81.
[20] Semaine Méd., 1897, **17**, 427.

of the digestive tract, in the peritoneum or abdominal viscera, in the larynx, the eyes, the nose, the ears, the kidneys, the genital organs, the nervous centers, in the bones or in certain muscle masses clinical and experimental evidence are in agreement that this rule is absolute. The chapters to follow will furnish an abundance of proof.

Of the autopsy observations, Biedert's figures on the principal localizations of tuberculous infection will be found sufficiently accurate. They summarize those already published by Simmonds, Rilliet and Barthez, Steiner and Neureuther, Widerhofer, Steffen, and others and are based upon 3104 autopsies of adults and 1346 of children. They may be condensed as follows:

LOCALIZATIONS	ADULTS	CHILDREN
	per cent	*per cent*
Pulmonary lesions in	91.2	79.6
Intestinal lesions in	40.7	31.6
Glandular lesions in	26.8	88.0*
Peritoneal lesions in	18.0	18.3

* 78 per cent bronchial, 10 per cent mesenteric.

CHAPTER VIII

MECHANISM OF TUBERCULOUS INFECTION

Penetration of the Virus into the Body by Way of the Skin and Mucous Membranes

A knowledge of the mechanism by which the tuberculous virus penetrates into the body of man and susceptible animals is of extreme importance, since it must serve as the basis for both individual and collective measures of defense against the spread of the disease. Therefore it has been the object of a great deal of study from both the clinical and experimental standpoints.

To thoroughly understand this mechanism one must have in mind the essentials of what we know today regarding the anatomy and physiology of the lymphatic circulation. It will not be amiss then to briefly recall these subjects at this point.

A. LYMPHATIC CIRCULATION, LYMPH, GLANDS, RÔLE OF THE LEUCOCYTES IN TUBERCULOUS INFECTION

All the organs possess a system of lymph capillaries which penetrate and anastomose within the meshes of the connective tissue, establish communications between them and the serous cavities and form small lakes and widened spaces. This capillary network is lined with a single layer of endothelial cells; it drains the lymph (or interstitial fluid) in which the tissues are bathed, and collects it into the lymphatic vessels which conduct it to the lymph nodes.

The lymph, produced throughout the body by cell metabolism,—and in particularly large quantity in the abdominal viscera,—contains chiefly, in addition to a basal fluid substance analogous to blood plasma (but more watery and less rich in albuminoid material), white cells or *leucocytes*. These cells differ in number according to the animal species (about 7200 per cubic millimeter in man; 7500 in the dog; 11,300 in the rabbit and only 180 in the frog).

The leucocytes are *motile* after the manner of the *amebae*. They put forth protoplasmic extensions, at times rounded, lobate and broad,

again filiform and slender. By virtue of their motility they are able to penetrate the endothelial cells of the lymphatic capillaries, to enter into the blood capillaries or to leave them and scatter about in the tissues, to migrate to the cutaneous or mucous surfaces and into the interior of the alimentary tract. Like the amebae, they have the faculty of ingesting solid particles and cellular or bacterial debris. They can even attack and absorb degenerated cells.

There are several varieties which are found in unequal number in the lymph. Some (the *lymphocytes*), of which the diameter is equal to or a little less than that of the red blood cell (5 to 8 microns), are round or oval, with a central nucleus filling almost the entire cell and leaving a very narrow margin of protoplasm. These lymphocytes contain no granules and are incapable of ingesting foreign bodies; they are therefore not *phagocytes* (Metchnikoff). They are only slightly motile. In human blood they constitute about 31 to 25 per cent of the white cells. They are rather more numerous in children and in adults during digestion, less numerous in the aged.

A second variety of leucocytes is made up of the *medium* and *large mononuclears* (lympholeucocytes of Pappenheim). They differ greatly in diameter, varying from 10 to 25 microns, are round or irregularly oval and contain a large kidney or horse-shoe shaped nucleus, often divided into two lobes. They are markedly phagocytic, ingesting altered leucocytes, degenerated tissue cells and certain bacteria such as the *Bacillus leprae.* Their proportion in the blood is relatively small: 4 to 8 per cent of the white cells. Their ameboid movements are rather sluggish and they end by becoming fixed in the connective tissue.

The *leucocytes with neutrophile granules*, the so-called *polynuclears*, or more exactly the *polymorphonuclears*, constitute a third and more numerous variety (40 to 75 per cent, average 60 per cent). They are 10 to 14 microns in diameter and have a polylobed nucleus, of quite variable shape, which is formed of 2 to 4 irregular masses joined one to another by fine filaments, and which takes a dark color with the triacid stain of Ehrlich (mixture of methyl-green, methyl-orange and acid-fuchsin). Their protoplasm is studded with neutrophile granules which stain violet. These cells, which are very motile, readily phagocytize bacteria and in particular the bacillus of tuberculosis.

The cells with acidophile granules, or *eosinophile myelocytes*, make up a fourth variety. They also have a polylobed nucleus provided with cytoplasmic granules. Their protoplasm contains an abundance of granules which color intensely with the acid stains, eosin or orange. They are also phagocytic, but to a less marked degree. They constitute 2 to 4 per cent of the white cells of the blood and are found to be considerably increased in a wide variety of diseases.

A last variety of leucocytes is represented by the *cells with basophilic metachromatic granules* (mastzellen of Ehrlich). These cells are round, polygonal or tapering, at times even branching. In diameter they are from 8 to 12 microns and are found in only very small numbers in human blood (0.05 per cent). They also contain granules which stain by *Gram* and *Ziehl*; at times also small vacuoles. The nucleus, which is always very large in proportion to the protoplasm, takes a pale blue tint with Unna's polychrom blue. They appear to have no phagocytic function and they become more abundant in the course of certain pathological conditions.

These various white cells, carried about by the lymph in the lymphatic capillaries, fill the *sinuses* of the *lymphatic glands*. They enter in by the vessels afferent to the *convexity of the glands* and there undergo multiple transformations. It is in these glands that the lymphocytes, and probably also the large mononuclears, are generally formed. There too the eosinophiles are produced, and perhaps also the polymorphonuclear neutrophiles which accumulate in the glands in great number, bearing innumerable particles and bacteria phagocyted before their arrival. It is there that the processes of intracellular digestion are chiefly affected, and the lymph node, thanks to its delayed circulation, serves not only as a sort of filter but also as a laboratory wherein is elaborated a host of protein, carbohydrate and fat splitting ferments (trypsin, amylase, lipase, etc.).

The younger the individual, the more active is this laboratory and the more efficient the filter. Little by little, with increasing age, the glandular tissue undergoes sclerosis, especially at the hilus (Salimbeni and L. Géry[1]); the capsule thickens, the connective tissue framework hypertrophies, and in the pulp the lymphocytes become more and more rare, while the macrophages predominate, many of them containing yellow pigment and having a very acidophile protoplasm.

Whenever a tubercle bacillus, deposited upon skin surface or

[1] Ann. de l'Inst. Pasteur, 1912, 26, 577.

mucosa, or introduced into the healthy body in any other way (inhalation, traumatism), finds itself near a motile polymorphonuclear leucocyte, it immediately becomes the prey of the latter which takes it along in the lymphatic or blood circulation. The digestive ferments of the leucocyte do not succeed in digesting the bacillus because, as we have seen before, it is protected by an extremely resistant fatty waxy ectoplasm. The bacillus remains enclosed in its phagocyte or, if rejected by the latter, is immediately ingested by another which in turn transports it for a longer or shorter time in the circulation. This continues until such time, *often remote*, as the leucocyte host, poisoned and then degenerated and killed by the secretions of its parasite, becomes the prey of one of the large mononuclears which pave the endothelium,—for it is one of the essential functions of the large mononuclears to absorb and digest degenerated and dead cells.

A little later, this mononuclear too will be subjected to the effects of the toxins which will have increased in the cell with the multiplication of the original bacillus. These effects will soon manifest themselves by that peculiar pathological formation which we have studied under the name of the *giant cell*, the first stage of the *tuberculous lesion*.

But between the moment when the virulent germ has affected its entrance into the body and the beginning of the formation of this first tuberculous lesion, an often considerable interval of time may have elapsed, which the infected organism has utilized to set at work its defensive reactions and its means of elimination.

Thus the defensive reactions, in a great many cases, come into play with sufficient success to wall in, as it were, the focus of infection by a mass of large mononuclears which become organized into dense connective tissue, either in a lymph node sinus or in a lymphatic or venous capillary of the lung or of some other organ. The lesion then remains *latent* and may continue so during several years.

In other cases, still more fortunate, one or more bacilli phagocyted by motile polymorphonuclear leucocytes are evacuated from the body through the liver and intestines, as are pigment granules or inert foreign bodies before they have been able to initiate the formation of a giant cell lesion at a point of arrest (*lésion d'arrêt*).

When the infection is produced by very virulent bacilli, that is to say by those which are perfectly adapted to the invaded host, and when these germs are present in a large number, or cause, though attenuated, a massive infection, the normal processes of defense and

elimination are no longer able to perform their protective function and the fact of this inability is soon manifest in the group of lymphatic glands nearest to the portal of entry of the virus. Then,—and then only,—the law of Cohnheim, of which I spoke in the preceding chapter (VII, B), holds good. The leucocytes engorged with bacilli and rapidly poisoned, become arrested in the cavernous sinuses of the first lymph node encountered on their way, and there they succumb. Their debris (and virulent contents) become the prey of mononuclears which quickly organize themselves into giant cells and the same lymph node becomes the seat of several tubercles whose later caseation will result in the pouring of a relatively considerable number of bacilli *into the efferent lymph channels.* Through the latter the bacilli will go elsewhere to create other foci of tuberculosis, near-by or at a distance.

Such is the mechanism of infection by the tubercle bacillus. One will readily understand the enormously important rôle played therein by questions of *quantity* and *source of the virus,* and also by the anatomical structure of the organs into which the virus is borne by the lymph or by the blood.

B. PORTALS OF ENTRY OF THE VIRUS IN LATENT TUBERCULOUS CONDITIONS

It seems at once obvious that *in cases of latent infection,*—the most frequent form, as attested by the very large proportion of apparent healthy human or bovine subjects who react to tuberculin,—it is quite impossible to disclose the route followed by the virus in its original invasion of the body. Whatever may have been the portal of entry of the infection, it closed itself behind the migratory leucocyte which phagocytized the bacillus, without the slightest trace of the passage remaining. The long wanderings through the lymphatic channels, then into the blood stream, remain equally masked, and only when the virus causes somewhere the formation of a tubercle, can its presence, until then unrecognized, be revealed by a positive tuberculin reaction.

These cases of latent infection, brought about by very small numbers of bacilli or by those less virulent—that is to say not adapted to the organism invaded,—are observed with extreme frequency in man and in animals spontaneously susceptible to tuberculosis.

Orth had already noted them in 1876; but it was chiefly Loomis[2] as the first (1890), followed by Pizzini (1892) and Kälble (1899) who attracted attention to them by proving that bronchial or other glands, even though normal on microscopic examination, and removed from subjects showing no sign of tuberculosis, nevertheless contained bacilli.

Since then, the investigations of A. MacFadyen and MacConkey,[3] of Harbitz, of Weichselbaum and Bartel, of Rosenberger (1905), those which I published with C. Guérin and A. Deléarde in 1906,[4] those of L. Rabinowitsch (1907), of S. Arloing (1909)[5] and still others, show that in man, in early as well as in adult life, and also in cattle, *tubercle bacilli are frequently found in the mesenteric, mediastinal and tracheo-bronchial lymph glands, where there exists no lesion nor any suspicion of tuberculosis.* Harbitz systematically inoculated into guinea pigs the different lymph node groups from 91 non-tuberculous children, he obtained positive results 18 times with cervical, bronchial, mesenteric and retroperitoneal nodes.

With C. Guérin I have shown that, in the bullock infected simply through living with other animals, tubercle bacilli may remain latent over a very long period (more than 11 months) without producing any lesion in the lymphatic system.[6] Furthermore our experiments, coming after those of Schroeder and Cotton, of Rabinowitsch, of Ravenel, and of Moussu, proved to us that apparently healthy cows, free from udder lesions but reacting to tuberculin, eliminate virulent tubercle bacilli from time to time either in their dejections or in their milk.

In human beings and in animals which harbor these latent tuberculoses, it is therefore never possible to indicate precisely either the manner of infection or the portal of entry of the bacilli, or even the time of their penetration into the body. There is reason to suppose that, in the majority of cases, the penetration is brought about through the medium of intestinal absorption. Indirect proof is furnished by the fact, pointed out some time ago by Nocard, that, during digestion, many microbes emigrate from the intestine with the chyle and are

[2] J. Am. Med. Assn., 1891, **16**, 98.

[3] Brit. M. J., 1903, ii, 129.

[4] Compt. rend. Acad. des sci., 1906, **142**, 1136.

[5] J. de méd. vét. et zoötech., 1909, 5. s , **13**, 193.

[6] Compt. rend. Acad. des sci., 1913, **156**, 34.

recovered again, first in the mesenteric glands, next in the lymph of the thoracic duct, then in the blood and in the majority of the organs (liver, spleen, kidneys, bone marrow, muscles, etc.). Culture of lymph, or of blood or pulp from these organs, during the hours which follow digestion, frequently gives a growth, whereas none occurs if the material is taken when the animals are fasting. If the organisms have disappeared at the latter time, it is because the bactericidal action of the body fluids and phagocytosis have had time to act. But where the invading organism is the tubercle bacillus we know that these defensive processes, particularly phagocytosis, are relatively inert.

To be sure, the intestine is not the only source of physiological infection of the tissues. It may also arise from the lungs. Nevertheless the rôle of this organ is incontestably much less important, first because the respiratory channels are less charged with bacteria than is the digestive tract, and furthermore because the mucus and ciliated epithelium form obstacles which are overcome only with difficulty. Besides, if physiological bacterial infection of the body fluids is commonly effected by way of the lungs, inoculations of blood and different tissues would give positive results aside from during the period of digestion, and we have seen that such is not the case.

C. TUBERCULOUS INFECTION THROUGH THE SKIN

Of all the organs of the human body, the skin offers the least favorable conditions for the retention, penetration and multiplication of the bacilli, as clinical observations demonstrate. Moreover, despite the frequency of contact of the integument with infectious material (sputum and bacillus-containing excretions), cutaneous tuberculosis is rare and, when it occurs in one of its forms (lupus, tuberculous ulcer, verrucous tuberculosis, scrofuloderma, anatomic tubercle), it tends generally to remain benign and localized, and to evolve slowly toward a chronic course or toward spontaneous healing (*see Chapter XVII*).

Nevertheless, grave and rapidly progressive forms of infection are sometimes observed following contamination of an accidental or surgical wound by excrement or by saliva containing bacilli.

In young calves, one encounters, not rarely, a tuberculous ulceration of the navel resulting from contamination of the umbilical wound by infected bedding.

PLATE V

1. Generalized tuberculous infection in the guinea pig. (Infection via digestive tract with human tubercle bacilli.) Caseous tubercles in the lungs, the liver, the spleen, the tracheo-bronchial and sub-lumbar glands.

2. The lymphatic stage of the infection produced by instillation upon the ocular conjunctiva, in the guinea pig. (Primary adenopathy of the cervical and tracheo-bronchial lymph nodes. Progressive extension of the tuberculous lesions by way of the lymphatics to the lungs and to other viscera).

II

I

Among the poorer Jewish or Mohammedan children, ritual circumcision, uncleanly done, becomes from time to time the point of departure for a fatal tuberculosis. L. E. Holt, of New York, was able to collect from the medical literature 41 such cases, 17 of which, to his knowledge, ended fatally. Some of them survived 11 months, to die of meningitis, but the usual termination of this form of infection was generalized glandular and visceral tuberculosis.

It has been reported that several children have been infected by one and the same operator as in the 10 cases reported by Lehmann.

Experimentally, it is proved that tuberculous infection can be effected through healthy skin after vigorous friction (that is to say under conditions which favor the migration of leucocytes from the superficial lymphatic network to the interstices of the epidermal cells), —and particularly skin which has been depilated or newly shaven,— by smearing upon it either bacillus-rich sputum or virulent cultures. Babès and Riegler, J. Courmont and Lesieur, Carl Fraenkel, H. Takeya and Dold[7] have thus produced tuberculosis in guinea pigs, rabbits and cattle. These authors have established that transcutaneous infection, usually sluggish, may leave no trace, nor develop local adenitis as a primary symptom, and may go on meanwhile to generalized disease. The proportion of positive results differs according as the skin is broken (75 per cent) or apparently intact (25 per cent). At times, particularly in the rabbit, the virus localizes itself in the lungs without setting up cutaneous or glandular lesions which can be demonstrated either macroscopically or microscopically. Here we have an example of pulmonary tuberculosis which originates at some distance from the respiratory tract, and whose portal of entry can not be discovered.

At other times, especially if attenuated bacilli are employed, skin alterations *in situ* are produced similar to those characteristic of verrucous cutaneous tuberculosis or scrofulo-tuberculosis in man, and having the same tendency to heal spontaneously.

D. INFECTION THROUGH THE MUCOUS MEMBRANES (OCULAR, NASAL, BUCCOPHARYNGEAL, GENITAL)

At the mucous surfaces, about the points where the glandular acini pour out their secretions, the lymphatic vessels and sinuses

[7] Arb. a. d. Geb. d. path. Anat. . . . . Inst. zu Tübing., 1908, 6, 710.

form a particularly dense network and the wandering leucocytes perform one of their chief functions, namely the sweeping from the mucous membranes of all bacterial or other impurities brought there by the external air.

Moreover these mucous membranes are particularly exposed to infection by the tubercle bacillus, or to be more exact, they offer an easy path for the penetration of the virus into the subjacent lymph spaces.

a. Ocular mucous membrane

As I have demonstrated with C. Guérin and V. Grysez,[8] if a particle of tuberculous sputum or a drop of a culture (containing for example 0.01 mgm. of virulent bacilli) be allowed to fall upon the eye-ball of a guinea pig, it suffices to set up a typical glandular tuberculosis in that animal, *without producing the slightest lesion in or about the eye.* Such a glandular tuberculosis, beginning in the retromastoid gland, quickly invades the two retropharyngeal glands, the two glands of the anterior part of the neck, then the tracheal and bronchial glands, and extends in the course of 4 to 5 weeks to other visceral groups, to the glands of the liver hilus, to those of the mesentery, at times to superficial glands like the inguinal, to the spleen and almost constantly to the lungs.

This mode of infection is, therefore, very certain and, aside from its severity (due to the virulence of the bacilli employed), the resulting form of tuberculosis is, in its first phase, singularly like that which characterizes *scrofula* in man, especially in childhood. Such is the resemblance that, on examining experimental animals, one is immediately struck with the idea that human family contagion probably frequently takes the same path and that it then follows the projection, by a tuberculous cougher, of particles of saliva abounding in bacilli upon the ocular conjunctiva of healthy individuals.

Infection by ocular instillation, producing as it does a sort of natural infection by the lymphatic channels without destruction of tissue and *without lesions at the portal of entry of the bacilli,* enables one, much better than does experimental inoculation, to study in the guinea pig the action of tuberculins, sera and chemical substances capable of influencing the progress of the tuberculosis. This pathway

[8] Compt. rend. Soc. de biol., 1913, **74**, 310.

may also be utilized to advantage for attempts to vaccinate with modified or attenuated bacilli.

Spontaneous tuberculous infection of the eye is observed but rarely in man and cattle. It is scarcely ever encountered except in individuals who at the same time have tuberculosis elsewhere and where found it is as a complication of generalized tuberculosis. The choroid, the iris, or the anterior chamber, may be the seat of a tuberculosis of vascular origin. The conjunctiva and the cornea are sometimes infected by the hands soiled with sputum, or through small abrasions, the individual being phthisical. Lesions so produced are always accompanied by swellings of the preauricular glands, those at the angle of the jaw or of the cervical glands, and these engorgements are then the indicators of the nature of the disease. They are observed frequently for example in *phlyctenular conjunctivitis,* which is of tuberculous origin.

b. Nasal mucous membrane

The *mucosa of the nose,* despite the richness of its lymphatic and venous supply and in spite of the enormous amount of dust of all kinds which accumulates upon it at each inspiration, is not penetrated by the tubercle bacillus as readily as one might assume. The reason, which is easily understood, lies in the fact that the dusts,—bacterial, vegetable or mineral,—exercise for the most part a positive chemotaxis toward the leucocytes, which by diapedesis go forth from the submucous lymphatic capillaries and spaces to phagocytize them. Many of the leucocytes however find themselves entrapped at the mucous surface in the folds of the turbinates, by a particular variety of sticky mucous secretion; they then become incapable of again passing through the mucous membrane to reenter the circulation, and the only fate remaining to them is to be expelled from the nose.

The proof that events happen thus lies in the fact that bacilli are frequently found in the nasal cavities of perfectly healthy individuals. By 1894 Strauss had already demonstrated the fact by examinations of non-tuberculous patients and students attached to his service at the Charité Hospital at Paris. From these different subjects he collected the dust, the crusts and the mucous discharges contained in the nasal cavities, by means of small previously sterilized cotton swabs. The swabs were stirred about in a test tube of broth and

sterile water and the very turbid liquid thus obtained was injected into the peritoneum of guinea pigs. Of 29 subjects, there were 9 who harbored virulent bacilli in their nasal passages.

Le Noir and J. Camus[9] in repeating these experiments collected their material from the nasal cavities of the hospital personnel, physicians, students and nurses, in a ward in which were 14 tuberculous cases. This was done after the ward had been twice swept, the beds made, the bed tables dusted, etc. The cotton tampons covered with the mucous secretions were inserted under the skin of 9 guinea pigs by means of small incisions.

Not one of the pigs became tuberculous.

The same tests repeated with nasal mucus from the tuberculous patients themselves, all of whom had bacilli in their sputum, gave only 3 positive results in 13 cases.

The nasal cavities constitute without doubt an excellent agent for filtering air and by means of the mucus there secreted in abundance they retain the greater part of the bacteria, prevent them from penetrating further, and expel them.

On this account they are only exceptionally the seat of lesions of primary infection. F. Fraenkel has never seen a single case. They are reported however in the medical literature, although they are extremely rare and are always accompanied by the characteristic engorgement of the glands of the neck.

On the other hand, secondary infection of the nose in the form of lupus ulcers or of tuberculous ulceration is encountered fairly frequently in phthisical patients, who have probably re-infected themselves locally with fingers soiled with bacillus-containing sputum. This secondary nasal infection is at times, especially in children, the point of departure for a tuberculous meningitis, through propagation by the lymphatics over the cells of the ethmoid bone (Naunyn and Schreider, Demme, Schwalbe and Flatau).

c. Bucco-pharyngeal mucous membranes—Tonsils

The mouth and the pharynx oppose the direct penetration of tubercle bacilli into the subjacent lymphatic system with the same natural obstacles as do the nasal cavities. The leucocytes, which migrate by diapedesis from the submucous vessels, sweep the bucco-

[9] Compt. rend. Soc. de biol., 1908, **65**, 646.

pharyngeal cavity and are borne with the saliva to the digestive tract by the movements of swallowing, so that they do not reënter into the circulation with the bacilli which they have succeeded in ingesting.

Exception must however be made for that region of the naso-pharynx which is occupied by the *tonsils.*

These follicular lymphatic glands, made up of crypts and spaces tightly packed with lymphocytes and polymorphonuclear leucocytes, constitute, by virtue of their situation at the entrance to the digestive and the respiratory tracts, a very important system of defense against infections in general and tuberculous infections in particular. Many bacteria are there destroyed by the lymphoid cells.

Tubercle bacilli, as a rule, do not there meet such a fate. In most cases probably they are swallowed or expelled in the efforts of coughing, but it nevertheless happens that migratory phagocytes conduct them to the lymphatic circulation. From a massive tonsillar infection there can then result an engorgement of the retro-pharyngeal, sub-parotid or cervical glands.

Poirier and, after him, George B. Wood,[10] Robertson,[11] and A. Edmunds, have demonstrated that the lymphatics of the tonsil anastomose with the whole pharyngeal system tributary to the deep glands of the neck. The collecting vessels proper of the tonsil perforate the muscular coat of the pharynx a little above the large cornu of the hyoid bone and terminate in the nodes situated upon the internal jugular, just above the posterior belly of the digastric, where this muscle is crossed by the anterior border of the sternocleido-mastoid. This point is located a little behind and below the angle of the jaw.

When these glands are infected primarily by bacilli coming from the tonsils, the infection may extend to other cervical gland groups and propagate itself little by little to the tracheo-bronchial nodes, to the lungs and to other organs.

The primary lesion of tuberculosis may also, in certain cases, develop in the closed follicles of the tonsil and there progress to caseation and ulceration. Pressure with a tongue depressor on the crypt then forces out the caseous matter. The anterior pillars of the gland

[10] Am. J. Med. Sci., 1905, **130**, 216.
[11] J. Am. Med. Assn., 1906, **47**, 1725.

are red, tense, and congested, and the local engorgement extends to the sub-sterno-cleido-mastoid chain of nodes.

Tuberculous tonsils are usually neither large nor pedunculate, but on the contrary are small, pallid and closely set against the pillars. They do not occur frequently. John Wright, Hodenpyl, P. Nobécourt and Tixier[12] insist that they are rare. The lesions are localized only at the base of the gland, so that if one removes merely the superficial portion (an operation to be avoided) the portion most infected is left with the stump.

Tonsillar tuberculosis never remains limited to the tonsils which make up the *ring of Waldeyer*. It always extends almost simultaneously to the pillars, to the soft palate, and to the posterior walls of the mouth, but it predominates in one gland or another. It is most often encountered, not as a primary lesion, but as a secondary localization in phthisical and other forms of tuberculosis. It tends to form an irregular ulceration, more or less concave, extensive, and granular at the base (A. Hautant).[13]

In the bullock, primary tuberculosis of the tonsils is exceptional. According to Max Devriendt,[14] who has collected important data on the subject at the abattoir at Berlin, infection of these glands appears only secondarily in animals which are already possessed of other glandular or pulmonary lesions and is said to follow upon the repeated ingestion of tubercle bacilli.

d. Genito-urinary mucous membranes

Tuberculous infection of the genito-urinary tract is most often of hematogenous origin. It may result also from a direct infection of the mucosa (vulvar, vaginal, urethral or bladder) by sexual relations or by the introduction into the genital organs of catheters, irrigation tubes, sounds, fingers or other objects contaminated with bacilli. Verchère, Fernet, and Derville,[15] have published several observations relative to wives who contracted pelvic peritonitis from contact with husbands suffering from pulmonary or epididymis tuberculosis.

[12] Gaz. des hôp de Par., 1908, **81**, 1287.
[13] Rev. de la tuberc., 1906, **3**, 326.
[14] Deutsch. tierartz. Wchnschr., 1909, **16**, 729; 745.
[15] Thèse, Paris, 1887.

Primary localizations in the vagina occur only very exceptionally. According to Cornil, this is due to the fact that the thick stratified pavement epithelium which lines the vagina renders it difficult for bacteria to penetrate as deeply as the encompassing lymphatic spaces, despite the marked development of the latter. But if some leucocytes in their migration do carry in a few bacilli, these microörganisms will settle in the sub-pelvic system and there give rise to tubercles among the viscera of the pelvis.

The fact that tuberculous salpingitis and metritis have been reported in young virgins, and also tuberculosis of the testis in young boys, is proof that such localizations may result from blood infections.

Experimentation shows nevertheless that primary infection of the genital tract may be easily brought about. Thus Cornil and Dobroklowski[16] introduced a few drops of a culture of tubercle bacilli into the vagina of a female guinea pig, being careful not to wound the mucous membrane. As early as the fifteenth day thereafter microscopic examination revealed the presence of minute tubercles in the uterus, beneath the intact epithelial lining.

Gaertner[17] inoculated the virus directly into the testicles of guinea pigs and rabbits and demonstrated that a certain number of females impregnated by these animals, became tuberculous, with vaginal and uterine lesions; and Baumgarten produced an ulcerative tuberculosis of the posterior urethra and of the neck of the bladder by instilling bovine bacilli into the urethra of male rabbits.

In my laboratory, M. Breton[18] readily succeeded in producing primary infection of the guinea pig bladder by directly introducing bacilli from a culture. There was thus produced, on the walls of the cavity, a granular infiltration which ulcerated and extended progressively to the sublumbar and retromesenteric glands, then to the tracheo-bronchial nodes and to the lungs. In such cases, the kidney always escaped.

Clinically, tuberculosis of the bladder is extremely rare. When observed it is almost always secondary to a renal or prostatic lesion (*see Chapter XV*).

[16] Internat. Congr. on Tuberculosis, Paris, 1888.

[17] Ztschr. f. Hyg., 1893, **13**, 101.

[18] Ann. de l'Inst. Pasteur, 1910, **24**, 820.

CHAPTER IX

TUBERCULOUS INFECTION BY THE RESPIRATORY PASSAGES

The extreme frequency of localization of tuberculosis in the lungs and the fact that it often exists to the apparent exclusion of all other localizations in a certain measure justified the opinion which has prevailed until very recent years, that the tubercle bacillus usually penetrates into the body by way of the respiratory passages. Today however we possess more definite knowledge of the various processes of infection by the tubercle bacillus. Careful clinical observation and experimentation have led us to the conviction that the infection most frequently remains from the outset and for a long period,—sometimes even indefinitely,—concealed (*occulte*) in the lymphatic glandular system before inducing the formation of *tubercles* capable, by their development and later caseation, of discharging bacilli into the lymphatic or blood circulation. Therefore we are obliged to examine the question more closely and to modify our interpretation of facts which up to now have been incompletely studied.

A. MECHANISM OF PRIMARY TUBERCULOUS INFECTION OF THE RESPIRATORY PASSAGES

Primary tuberculous infection of the lung or of the bronchi may be brought about either by way of the air passages, that is to say directly by bacilli borne by the air entering into these organs, or by the blood stream. In the latter event,—*more frequent by far*,— some leucocyte, containing its parasitic bacilli recently introduced into the body or derived from a more or less long standing focus of infection, is arrested in the interalveolar or peribronchial capillaries and becomes the point of departure for the formation of a giant cell.

The disposition of the bronchial tree and the character of the mucous membrane with which it is lined from the epiglottis onward presents quite special characteristics which tend to assure the protection of the lung alveoli against so deep a penetration of mineral, vegetable or bacterial dusts suspended in the inspired air.

Within the larynx, the mucosa is covered in parts with stratified squamous epithelium (anterior surface of the epiglottis, the true vocal cords and small areas in the aryteno-epiglottic folds), and in other parts with a stratified cylindrical ciliated epithelium. The latter is traversed by numerous excretory ducts from mucous glands of alveolar or ramified tubular type, and encloses, especially at the level of the ventricles, true closed lymph follicles (*tonsillae basilares*) from which wandering leucocytes are constantly issuing forth. These closed follicles communicate at their bases with the lymphatic vessels and spaces of the submucosa, which discharge, either into a gland placed between the greater cornu of the hyoid bone and the superior border of the thyroid cartilage (Treichmann), or into the glands situated beneath the sterno-mastoid muscle at the level of the bifurcation of the common carotid (Sappey), or again into the glands situated at the two sides of the membranous portion of the trachea (Treichmann).

The tracheal epithelium and that of the bronchi are also formed of ciliated cylindrical cells, with little islands of flat stratified cells through which the excretions of numerous mucous glands are poured out. The very extensive subjacent lymphatic system is distributed in two layers, one superficial, the other more deep, which pass together toward the chain of glands strung along each side of the trachea and of the esophagus.

As for the lung itself, it is known that it can be subdivided into a number of segments or lobules, each one of which in a sense represents the expansion of a bronchial branching divided into bronchioles which terminate in alveoli or vesicles. "The lung," says Laguesse, "is a hollow tree ramifying almost to infinity, whose numerous branches are the bronchi, whose ultimate twigs or alveolar canals widen themselves, form alveoli and change their structure in order to assume the character of respiratory surfaces and to adapt themselves to the function of blood aeration."

The number of alveoli in the human lung is enormous. Aeby says that there are about 404 millions of them in the adult, representing a surface of 79 square meters in average inspiration when resting and of 129 square meters in a state of complete dilatation. Indeed, according to researches of Léon Bernard, A. Le Play and Ch. Mantoux,[1] one-sixth of this surface is adequate to support life.

[1] J. de Physiol. et de Path. gén.. 1913, **15**, 16.

Each lobule, the average volume of which is about one cubic centimeter, is separated from its neighbors by connective tissue partitions which enclose lymphatic spaces and which are very compact and resistant, particularly in the adult and still more so in the aged. The epithelium of the penetrating bronchus there loses its cilia, becomes cubical, and transforms itself into small nucleated granular cells, flat, rounded or polygonal, and into broad lamellae or plaques without visible nuclei (cells of Koelliker), which cover particularly the alveolar walls over the edges of the separating partitions. These cells, whether small or large, lie upon an extremely thin fibrillar membrane, reinforced by elastic and smooth muscle fibers.

Many alveoli communicate with one another by means of pores which Henle, Hansemann, F. E. Schulze, and more recently E. Laguesse and R. Marchand[2] have demonstrated. These pores, ordinarily very small, become expanded in certain pathological conditions of the lungs, principally in emphysema.

The epithelial cells of the alveolar wall are constantly desquamating and collecting in the cavity, along with many leucocytes which issue by diapedesis from the interior of the vessels. When an inflammatory process is set up by any irritant foreign body borne in by the air (tubercle bacillus for example), these migratory dust cells (*cellules à poussières*, *Staubzellen*) are found in great abundance throughout the exuded serous fluid which fills the cavity of the alveolus. At times they are carried on toward the bronchi and swept out of the lungs by expiration and the movement of the cilia of the bronchial epithelium, or they may reenter the perialveolar lymphatic circulation and be carried to the nearest glands.

In the denser portion of the connective tissue partitions which separate one alveolus from another the blood capillary system is spread out. The latter projects into the cavity and is so dense that the diameter of the acini is scarcely equal to that of a red blood cell; with the result that the surface of each alveolus is overspread with an almost continuous sheet of circulating blood, separated from the air by an extremely thin single layer of epithelial cells.

In miliary tuberculosis, the initial lesions take rise precisely in these vessels, the diameter of which is insufficient for the passage of a

[2] Compt. rend. Soc. de biol., 1911, 70, 178.

leucocyte engorged with bacilli and rendered incapable of movement. The resulting irritant embolus then serves as a location for the formation of a *giant cell.*

About each alveolus and each lobule, the abundant lymphatic spaces and vessels drain the lymph and carry it to the glands of the lung hilus by way of the collecting peribronchial and perivascular trunks, some of which are superficial and subpleural, while others are more deeply placed.

In the bullock, each lobule is surrounded by wide partitioned lymphatic spaces which are paved with a wavy epithelium (Pierret, Renaut, Sussdorf) and become engorged with serous exudate in certain pathological conditions (*péripneumonie*).

In air-produced tuberculous infection, the wandering leucocytes issue from these peri-alveolar lymphatic spaces and play the principal rôle. By causing mice or guinea pigs to inhale a small quantity of tubercle bacilli from a finely suspended culture and then killing the animals at intervals of 24 hours, 48 hours, three days, etc., and up to two weeks after the original inhalation, I could easily follow experimentally the evolution of the inflammatory process within the alveoli. Thus it was found that bacilli penetrating into an alveolus quickly induce there an influx of polymorphonuclear leucocytes and dust cells, all of which form at the center of the alveolus a mass which organizes into a small tubercle. This rapidly progresses to caseation and breaking down into pus leads to the extension of the caseous matter into the neighboring alveoli. The result is an infiltration which extends more or less to the whole of one lobule and then to several lobules. Meanwhile the wandering leucocytes have collected bacilli from the substance of this infiltration and have transported them through the perilobular or peribronchial lymph spaces and vessels as far as the corresponding glands of the lung hilus which in their turn become considerably swollen and the seat of tuberculosis.

The evolution of these primarily alveolar lesions, when produced experimentally, *is always rapid,* even in the large animals. Very often the pathological picture of *caseous pneumonia of children* is reproduced. But if the infection is small in amount, the bacilli ingested by the extravasated leucocytes, instead of giving rise to tubercles in the interior itself of the alveoli, reenter with the leucocytes into the lymphatic circulation and the initial lesions are formed only at the periphery of the lobule, or in the subpleural spaces, or again in the peribronchial glands.

It sometimes happens, and I have seen it occur in guinea pigs, that *a discrete airborne infection induces no intraalveolar lesion*, brings about the appearance of no primary lesion (*chancre d'inoculation*), nor of any pulmonary tubercle, and manifests its effects only more or less tardily by a glandular lesion at some point in the body relatively distant from the portal of entry of the bacillus.

In such cases it is impossible to detect any difference between the effects of an airborne infection and those which follow the direct penetration of the infecting element through other lymphatic channels.

An argument on favor of the predominance of airborne infection has been drawn from the fact that localizations of tuberculosis occur most frequently at the apices of the lungs, more particularly at the right apex. These localizations are encountered almost constantly following intravenous inoculations into animals, and result from purely mechanical causes and chiefly from the particular arrangement of the lymphatic vessels which permit a prolonged lymph stagnation. Bacmeister[3] performed some very conclusive experiments on this subject. He placed a metal band about the thorax of young rabbits, at the level of the first ribs, and as the animals developed the band produced a narrowing of the transverse diameter of the thorax and a constriction of the pulmonary and pleural tissue.

When, afterward, he injected an emulsion of bacilli into the marginal ear vein or into the superior vena cava of the rabbits, he regularly saw the tuberculous lesion first localize itself in and around the depression caused by the band. In the control animals nothing like this was produced; the tubercles were about equally scattered throughout the whole of the lungs.

Bacmeister *never succeeded in producing this localization in other similar animals when he caused them to inhale a fine spray of tubercle bacilli, despite the fact that the animals wore the band at the level of the first ribs.*

B. EXPERIMENTAL PULMONARY INFECTION WITH DRIED BACILLI OR WITH DRIED TUBERCULOUS MATERIAL

An early as 1869, Villemin[4] had shown that dried and pulverized tuberculous sputum can reproduce tuberculosis when insufflated

[3] Deutsch. med. Wchnschr., 1911, **37**, 1385.

[4] Gaz. hebdomadaire, 1869, p. 260.

into the rabbit's trachea. Later however, Cadéac and Malet[5] showed that dust collected in hospital wards occupied by phthisical patients, and sputa or fragments of tuberculous lungs dried and pulverized, produce tuberculosis only exceptionally by inhalation. Of 46 animals, rabbits and guinea pigs, which were made to respire several liters of these dusts for one hour each day during several weeks, only two became tuberculous.

In three later works (1898, 1905 and 1907),[6] the same investigators still insist on the difficulties encountered in communicating tuberculosis by inhalation of dried sputa. Of 38 guinea pigs and 11 rabbits which they tried to infect in a bell shaped funnel with an insufflator, 5 guinea pigs only developed the disease and two of these showed lesions indicating that the infection had occured through the digestive tract.

Other experiments proved to their minds that sputa dried in daylight are harmless and that those dried in the dark are capable of transmitting tuberculosis by inhalation only very exceptionally and then only when administered in massive doses. Practically speaking therefore, it must be admitted that *dusts contaminated with dry bacilli offer but little harm to healthy respiratory passages.*

Experiments were carried out at Pouilly-le-Fort in 1900, by Nocard and Rossignol, under the auspices of the Société de médecine vétérinaire and of the Société d'Agriculture de Melun, with a view to establishing the duration of the period of incubation of tuberculosis in cattle. Two heifers were infected by the inhalation of 3 cc. of dried and finely sifted tubercle bacilli from a culture; they reacted to tuberculin, one on the thirty-second day, and the other on the ninetieth day.

They were slaughtered soon after the test and their lungs as well as the bronchial and mediastinal glands were found full of miliary tubercles. The viscera and mesenteric nodes were normal in appearance; but proof of the absence of infection was not established by guinea pig inoculation. It was noted only that "the bronchi, the bronchioles and the pulmonary alveoli had escaped infection, and that tuberculous nodules were located under the pleura at the periphery of the lobules in the interstitial cellular tissue. Presumably, each tubercu-

[5] Rev. de méd., 1887, **7**, 337; Internat. Congr. on Tuberculosis, Paris, 1888.

[6] Rev. d'Hyg., 1905, **27**, 961; J. de méd. vét. et zootech., 1907, 5. s., **11**, 65.

lous focus had formed itself about a phagocyte which reentered into the lymphatic circulation after having ingested one or more tubercle bacilli."[7]

In order to settle the question as to whether dry virulent dusts can actually infect the alveoli, I performed the following experiment, in collaboration with Vansteenberghe:[8]

Two guinea pigs were placed under a bell jar inside of which a strong current of air, carrying a large quantity of bacilli, was made to pass for 20 minutes. The bacilli were of bovine origin, dried under a glass jar in the presence of calcium chloride only 24 to 48 hours at the longest, and then finely pulverized.

The two guinea pigs were killed and at once autopsied after this single period of inhalation, and different portions of their respiratory organs, the trachea, and the anterior and posterior lobes of the two lungs of each animal were inoculated separately and immediately into other guinea pigs.

Only the pigs inoculated with emulsions from the trachea and anterior lobes presented tuberculous lesions and the latter were very discrete. Those inoculated with the emulsion of the posterior lobes remained free from infection.

Therefore, despite the extreme method used to infect, only a very small number of bacilli succeeded in penetrating into the trachea and only as far as the first bronchial ramifications.

Numerous other experimenters (Baumgarten, Sirena, Pernice, Di Toma, Peterson) have vainly attempted to transmit tuberculosis by inhalation of dust containing live bacilli.

In the opinion of Köhlisch,[9] the guinea pig must inspire 50,000 dried pulverized bacilli in order to become infected.

Cornet in Germany and Kuss in France, on the other hand, affirm the virulence of dry bacilli.

Cornet[10] states that he succeeded in infecting a very large number of experimental animals with dust obtained by beating rugs contaminated with dried sputa. At the first attempt, 46 out of 48 guinea pigs were infected; and, by the thirtieth day, almost all at once, they showed miliary tubercles and lung cavities. The frequency of these

[7] Rep. pub. by the Soc. de méd. vét. pratique, 1901.
[8] Ann. de l'Inst. Pasteur, 1905, **19**, 787.
[9] Ztschr. f. Hyg., 1908, **60**, 508.
[10] Ibid., 1888, **5**, 191.

cavities would seem to indicate that the guinea pigs used were previously tuberculous. Later the same author repeated the test on 392 animals. Half of them died in a short time from septic conditions. Of 196 which remained, 59 became tuberculous and 137 remained uninfected.

According to G. Kuss,[11] sputa dried in darkness are still virulent after 15 days; but are no longer so after 19 days. In one series of experiments, he caused guinea pigs to breathe a mixture of powdered sputum and talcum for a period of thirty minutes to one hour. All of his animals died within two or three months with pulmonary and glandular lesions and generalized miliary tuberculosis. In another series, the pigs inhaled for one to three hours the dust from a carpet soiled with dried sputum, which was brushed for one minute every quarter or half hour. The animals died in from 40 to 86 days and at autopsy presented pulmonary or mediastinal lesions with extensive miliary tuberculosis of all the organs.

Chaussé[12] thought that bacilli contained in dried sputum are but slightly virulent after 24 hours and absolutely innocuous after 10 days. Five days at room temperature and only 12 hours at 37°C. are sufficient to deprive them of their power to infect.

His experiments tend to show that tuberculization of susceptible animals, such as the guinea pig, is better accomplished by brushing or shaking pieces of cloth or soiled linen in a tightly closed box. One to five minutes of breathing are sufficient.

C. EXPERIMENTAL INFECTION OF THE LUNGS WITH FRESH BACILLI AND WITH SPRAYS OF TUBERCULOUS MATERIAL

Although it is difficult to communicate tuberculosis to animals by making them inhale *dry* infectious dust, it appears that, on the contrary, the infection may be easily effected by the inhalation of fine *liquid* droplets, which contain tubercle bacilli in suspension.

As early as 1876 Tappeiner[13] had placed dogs, for a few hours each day, in a box wherin he ground up a large amount of sputum of phthisical patients, and in his animals had observed obvious lesions. In 1877 he extended his experiments with a better technique: he

[11] Compt. rend. Acad. des sci., 1908, **147**, 272; Bull. méd., 1908, **22**, 709.
[12] Ann. de l'Inst. Pasteur, 1914, **28**, 771; 1915, **29**, 556; 633; Thèse, Paris, 1916.
[13] Virchow's Arch., 1880, **82**, 353; Deutsch. Arch. f. klin. Med., 1881, **29**, 595.

mixed a teaspoonful of sputum from a case of tuberculosis with cavities in 300 to 500 gms. of distilled water and, by means of a steam atomizer, sprayed the liquid into a cage which was open on only one side, and in which was a dog. The animal was subjected each day to one or two inhalations of one hour duration. The number of inhalations and the period of stay in the cage were varied and the experiments continued 24 to 45 days. Twelve dogs were treated in this manner. In all, at autopsy the lungs were found full of tubercles: ten times in the form of miliary tubercles, once as a caseous pneumonia. The kidneys were usually involved; the liver and the spleen less constantly. Evidences of tuberculosis were present after the third week.

Thaon,[14] in 1885, exposed rabbits and guinea pigs for a quarter of an hour, morning and evening, during a period of one week, to a spray of ground-up tuberculous sputum suspended in water. By the end of the third week, the lungs of the rabbits were infiltrated with small gray tubercles and the guinea pigs died invariably in 12 or 14 days, with extreme dyspnea. At autopsy their lungs formed a bluish-red solid mass, peppered with yellow points. By killing a few animals successively from the outset of the experiment, it was possible to detect in the slides the arrival of the bacillus by way of the bronchioles, its penetration to the extremity of the respiratory passages and its multiplication in the pulmonary epithelium.

Cadaéc and Malet[15] had also observed that the infecting power of droplets when inhaled contrasts singularly with the difficulty encountered in producing infection with dried dusts. Of 45 guinea pigs made to inspire either fresh cultures of bacilli or finely triturated sputa, not one remained uninfected.

Nocard and Rossignol, in the already cited experiment at Pouilly-le-Fort, covered the heads of two cows with cloth bags and obliged them, during a period of six minutes, to breathe in 100 cc. of a fine emulsion from a culture of bovine tubercle bacilli. The two animals reacted to tuberculin after 13 and 19 days. At autopsy, their lungs were found infiltrated with a considerable number of miliary tubercles.

Another animal, however, into the trachea of which were injected 10 cc. of the same emulsion, did not react until the 38th day. At autopsy, one month later, there was not trace of lung lesions; the

[14] Compt. rend. Soc. de biol., 1885, **37**, 582.
[15] Rev. de Méd., 1887, **7**, 337.

mucous membrane of the trachea alone was studded with fine tubercles about the point of needle traumatism; the retropharyngeal glands, however, and the bronchial and esophageal glands were full of small tubercles. The bacilli therefore, despite their being introduced into the bronchi in enormous quantity, had been eliminated in the expectorated mucous discharges.

Vallée (of Alfort)[16] met with the same difficulties in infecting the lungs with bovine bacilli when he injected them directly into the trachea and when he sprayed a fine emulsion of ground-up bacilli into the naso-pharynx. Of 12 calves twice sprayed with 2 mgms. of bacilli, only 4 contracted tuberculosis. The lesions were confined exclusively to the retropharyngeal, cervical and tracheal lymph nodes, the lungs and tracheo-bronchial glands not being affected.

In order that the bacilli may surely penetrate as deeply as the alveoli, it seems necessary, as I showed with C. Guérin,[17] that they be projected as far into the trachea as the bifurcation,—that is to say into a zone not subject to the cough reflex,—by means of a flexible sound and in a fine state of suspension in a large volume of fluid. At once there is obtained a massive broncho-pneumonia, with miliary tubercles in whose formation the lymphatic vessels of the alveolar walls take part.

However, these are not at all the lesions which are ordinarily produced by the penetration into the alveoli of a few separate bacilli held in suspension in moist dust.

C. Flügge[18] (of Breslau) and his pupil H. Findel produced these lesions with the greatest ease, just as Preyss had already succeeded in doing (1891). And I have repeated their experiments many times with V. Grysez. Our method was to fasten guinea pigs or mice in a Reichenbach apparatus or in a still simpler one, like that shown in figure 9, and to make them inhale during varying periods of time quantities of bacilli which could be counted with sufficient accuracy and which were held suspended in the spray of a Buchner atomizer.

[16] Ann. de l'Inst. Pasteur, 1905, **19**, 619.

[17] Ibid., 1906, **20**, 609.

[18] Ztschr. f. Hyg., 1907, **57**, 104. In the work published by Flügge in 1908 (von Veit, Leipz.) under the title "*Die Verbreitungsweise und Bekämpfung der Tuberkulose*" will be found in detail the investigations of Flügge and his collaborators; Findel, Laschtchenko, Bruno Heymann, Ziesche, Neisser. Sticker, Beninde, Kölisch, and others. See also P. Chaussé, Thèse, Paris, 1916.

It was found that infection of the animals, accomplished now and then with a few bacilli (15 or 20, perhaps fewer), was more rapid and more intense in proportion to the number of bacteria inhaled. An average of 20 very virulent bovine bacilli suffice to produce in guinea pigs a miliary tuberculosis, with generalized lesions in all the viscera and fatal in 90 to 180 days.

The bacterial elements contained in a virulent suspension can be counted without difficulty by the following method: in 10 centigrams of a culture or of tuberculous material, triturated in an agate mortar, there are first carefully mixed 2 or 3 drops of ox bile, and then a

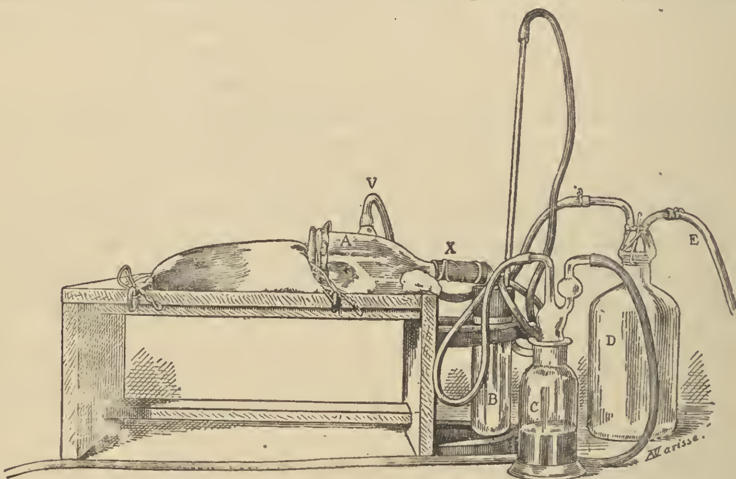


FIG. 9. SCHEMA OF APPARATUS USED TO INFECT THE GUINEA PIG BY INHALATION

measured quantity of physiological salt solution, 10 cc. for example. One drop of this dilution is placed upon a special slide which I had made by Stiassnie[19] and which contains a ruled area, each side being one half centimeter long and the whole divided into 25 squares. The surface of each square, therefore, represents 1/100 of a square centimeter. The drop is allowed to dry, the slide being kept horizontal. After the smear is fixed and stained with Ziehl by the ordinary technique the bacilli deposited upon each of the 25 squares are counted with an oil immersion lens and the total multiplied by 20 (number of drops per

[19] 204, Boulevard Raspail, Paris.

cubic centimeter). The result gives the average number of bacilli contained in one cubic centimeter of the dilution. *One milligram of culture weighed in the fresh state contains an average of 40 million bacilli.*

Although using a technique more or less different from that of Flügge or myself, numerous investigators of late years have produced direct infection in various animals (guinea pigs, dogs, cats, cattle) by making them inhale moist bacillus-containing dust. Some used sputa from phthisical patients (Hamilton and Young, Weber and Titze, Chaussé, in the calf, the dog and the cat; Kuss and Lobstein, in the guinea-pig); others preferred to use cultures of human, bovine or avian strains (Kossel, Weber and Heuss in the calf, Kuss and Lobstein, Louis Cobbett (of Cambridge) in the guinea pig, Weber and Bofinger in the mouse).

In 1907, H. Ziesche,[20] in Flügge's laboratory, was able to satisfy himself that some 30 to 40 per cent of phthisical patients eject in coughing, to a distance of 40 to 80 cm., droplets of saliva which are more or less rich in bacilli. These he collected in properly placed Petri dishes and was able to count the number of bacilli expelled, for example, during a half hour. In 20 per cent of the positive cases the number varied from 400 to 20,000 bacilli, while in 80 per cent fewer than 400 bacilli were found. It is evident that these bacillus-containing particles, which deposit themselves upon all sorts of objects (particularly on foods) and which at times contaminate the face or hands of those who are obliged to live in the immediate vicinity of these coughers and spitters, constitute a continual and most dangerous source of infection. The absorption of virulent elements so disseminated does not, however, in most circumstances, seem to be brought about by the respired air; it occurs much more readily and certainly through the mucous membranes, through the conjunctiva, through the mouth or by the digestive tract.

Chaussé,[21] like Sanger[22] before him, studied the conditions,—purely physical according to him,—which permitted the liquid particles to pentrate into the lung. He proved by some particularly ingenious experiments that it is only the particles from about 2 to 15 microns

[20] Ztschr. f. Hyg., 1907, **57**, 50.

[21] Compt. rend. Acad. des sci., 1913, **156**, 954, 1485; **157**, 862; 1914, **158**, 134;—Ann. de l'Inst. Pasteur, 1914, **28**, 608; 720; 1915, **29**, 556; 633;—Bull. de l'Inst. Pasteur, 1917, **15**, 33; 65.

[22] München. med. Wchnschr., 1901, **48**, 831.

in diameter which are respirable, since they remain in suspension in the atmosphere during a long period (up to 7 hours) and because they can be reflected by surfaces which arrest particles of greater volume.

According to this investigator, the passing of an air current over the surface of saliva or sputum, even at an initial velocity of 90 meters per second, separates out only a very small number of respirable particles. Of 31 guinea pigs which inhaled air blown under these conditions over the material containing the organisms, 600 litres of air having passed through the inhalation apparatus, but a single animal contracted one primary lung tubercle; but at this high velocity there was ruffling and breaking of bubbles.

With initial velocities of 35 meters per second or less, deep blowing of the sputum gave only a single tuberculous animal in 5 experiments on 33 guinea pigs. One guinea pig among twenty-two was infected with the velocity at 36.5 meters. Another experiment with a velocity of 80 meters per second was entirely negative.

Chaussé concluded from his researches that air contact at velocities of 30 meters per second or less can detach only a very small number of respirable particles from sputum or saliva; that, contrary to Flügge's idea, the majority of droplets borne away by the blowing process are too large to be transported and then inhaled, while those which are minute contain usually no bacilli.

No one denies, however, that bacteria suspended in very fine drops can, at times, penetrate by inhalation into the pulmonary alveoli. Experiments show that this is possible, that there results a rapid migration of leucocytes which phagocytize the bacilli, and that, according to the number and virulence of the latter, characteristic tuberculous lesions are soon formed, either *in loco* or *in the inter-alveolar or subpleural lymphatic spaces.* These lesions, primarily alveolar or peri-alveolar, extend somewhat later to the tracheo-bronchial nodes and very often, by blood or lymphatic channels, to other organs. This metastasis, as well as the fixation of infectious matter in the parenchyma of certain organs such as the lung, are greatly favored by the prolonged absorption of particularly noxious dusts (plaster, cement, mother-of-pearl, emery, etc.) as D. Cesa-Bianchi[23] demonstrated experimentally.

But it does not follow that this aerogenous path, *under normal*

[23] Ztschr. f. Hyg., 1913, **73**, 166.

conditions of natural infection, is the one by which man and susceptible animals are the most commonly and frequently infected.

D. CONDITIONS AND RELATIVE FREQUENCY OF PRIMARY INFECTION OF THE LUNG BY THE INSPIRED AIR

It should not be forgotten that the complexity of the respiratory passages (large filtering surfaces of the nasal cavities, pharynx, glottis, the length of the trachea and bronchi, and their lining ciliated epithelium), their reflex irritability which determines sneezing and the expulsion of offending particles, and the presence finally, throughout their length, of mucous-secreting glands designed to prevent extravasated leucocytes from reentering into the lymphatic circulation, render it exceedingly difficult for dust and infectious bacteria to penetrate directly as far as the alveoli, except under quite unusual circumstances where dusts and germs are extremely abundant in the respired air. These conditions are met with, as regards mineral or organic dusts, in certain industries (mining, metal working or textiles, for example) and they then induce anthracoses or pneumoconioses which, however, are usually harmless. They are also met, as regards tubercle bacilli, in the experimental conditions which are described above. But it is altogether improbable that tubercle bacilli, *floating isolated in the air, in the fresh state*, are often sufficiently numerous for many of them to escape the natural obstacles which the body opposes to their penetrating as deeply as the alveolus.

This improbability is the greater since, as numerous workers (Friedrich Muller,[24] Klippstein,[25] Bartel,[26] Boni,[27] Emmerich,[28] Quensel[29]), have shown, healthy lungs are almost always aseptic. And yet Saint-Clair Thomson and Hewlett[30] have shown that in London more than 14,000 microorganisms are inhaled per hour, and Hildebrand,[31] Lucien Beco,[32] and other authors cited in the lat-

[24] München. med. Wchnschr., 1897, **44**, 1382.
[25] Ztschr. f. klin. Med., 1898, **34**, H. 3/4.
[26] Centralbl. f. Bakt., 1898, **24**, 401; 433.
[27] Deutsch. Arch. f. klin. Med., 1901, **69**, 542.
[28] München. med. Wchnschr., 1902, **49**, 1610.
[29] Ztschr. f. Hyg., 1902, **40**, 505.
[30] Brit. M. J., 1896, i, 137;—Med.-chir. Tr., Lond., 1895, **78**, 239.
[31] Beitr. z. path. Anat., 1888, **2**, 411.
[32] Arch. de méd. expér., 1899, **11**, 317.

ter's work, have proved that the trachea of animals killed in their laboratories almost never contained any bacteria.

J. Arlo[33] demonstrated the same thing at the Pasteur Institute of Lille, when he cultured the lungs and tracheo-bronchial lymph nodes of a fairly large number of guinea pigs taken at random in the enclosures (where hay and straw were used for bedding, without any special precaution against dust) and killed by decapitation. He thus found that the right lung in 94.03 per cent of cases was sterile after 24 hours of culture and in 78.62 per cent after 48 hours of culture. The left lung was sterile under the same culture conditions in 99.02 and 79.02 per cent of cases respectively. The tracheo-bronchial lymph nodes on the other hand, were found fairly often infected or harboring bacteria which could be revived. They were sterile in only 52.28 per cent of cases after 24 hours and in 20 per cent after 48 hours.

J. Arlo concluded from his experiments:

1. That even in the very dusty atmosphere of the guinea pig enclosures, the air bacteria are arrested by the upper respiratory passages and reach only rarely the pulmonary alveoli.

2. That the bacteria which can be recovered in the lungs are present in only small number, since most frequently they appear in culture media only after 48 hours.

3. Finally, that in normal animals living under ordinary conditions, the tracheo-bronchial glands retain many bacteria caught by the leucocytes in the bronchi and carried into the lymphatic circulation, since, in about 50 per cent of cases, culture of these glands upon artificial media gives growth of saprophytic bacteria not yet digested by the phagocytic processes.

It is seen therefore that the defensive and eliminatory functions of the different structures guarding the entrance to the respiratory passages are conducted with wonderful perfection.

Furthermore, if primary infection in man were air-borne as frequently as many physicians today still think, and as P. Chaussé believes he has demonstrated,—without his experiments and arguments carrying conviction,—it would be incomprehensible why infection is so rare in animals readily susceptible to the human virus (the cat, the dog, the ass, the rodents of our homes or laboratories)

[33] Compt. rend. Soc. de biol., 1914, 76, 292.

and which breathe the air *ruminated*,—as Peter used to say,—by tuberculous individuals who throw off at times, according to B. Fraenkel, more than 7 billion bacilli in their sputum in a single day.

If, as Chaussé contends, *a single bacillus* penetrating into an alveolus is sufficient to set up a tuberculosis, it would be still less conceivable, considering the relative abundance and ubiquity of the tubercle bacillus, that one man or one susceptible animal in our hospitals or our cities or even on the face of the earth should escape the disease.

The fact is well established moreover that the air expired by phthisical patients contains no infectious elements. Tappeiner, and Grancher tried many times to infect animals by making them inhale, for a longer or shorter period, air which came directly from the respiratory passages of patients. They obtained only negative results.

The bacilli are contained only in the particles of saliva ejected in the efforts of coughing, and these are the minute droplets which Flügge, B. Heymann, and P. Chaussé[34] regard as the most active agents in the propagation of tuberculosis. Now, according to certain experiments of Chaussé, these liquid particles have a diameter of at least 30 microns and are not respirable! In his opinion they become infectious and capable of penetrating into the lungs only when in the dry state, and they dry very quickly in the atmosphere after their emission.[35]

Should it be said that primary air-borne infection is to be regarded as practically non-existent? That would be contrary to the evidence of facts which, although rare, seem well founded.

Among such facts, one of the most striking, because equivalent to a laboratory experiment, is that published by Reich[36] and bearing upon 10 new-born infants brought into the world by a phthisical midwife at Neuenburg. All of them died of tuberculous meningitis within 14 months, without there being any similar occurrence among children delivered by other midwives. The phthisical woman had the deplorable habit of practicing insufflation with the mouth, even when the infants showed no sign of asphyxia (Grancher and Hutinel).

[34] Ann. de l'Inst. Pasteur, 1916, **30**, 613.

[35] Soc. centr. de méd. vét., Mém. pour le prix Trasbot, 1912; Rec. de méd. vét., 1912, **89**, 555.

[36] Berl. klin. Wchnschr., 1878, **15**, 551.

One cannot help thinking that primary pulmonary infection occurs at times in a similar manner in very early life, for example, in the infant whose phthisical mother or nurse coughs before its mouth, the latter held open in search of the breast which is to feed it. Massive aerogenous infection can then produce intra or perialveolar primary foci as in the above-described experimental animals, and the always rapid evolution of the foci soon ends in the caseation and purulent softening which characterize the *caseous pneumonia of nurslings.*

But the problem is much more complex when we have to definitely establish the aerogenous origin of tracheo-bronchial adenopathies which are so frequent in children and which in a very large number of cases are the sole earliest indication of tuberculous infection.

Every one knows that Parrot[37] believed himself justified in asserting that there exists no tracheo-bronchial adenopathy that does not originate in the lungs. "Whenever a bronchial lymph node is the seat of a tuberculous lesion there is a tuberculous lesion of the lung." This "*loi des adénopathies similaires*" or law of similar adenopathies, based upon a large number of incontestably well-proved facts, defended by Hutinel, Hervouet, G. Küss,[38] Albercht, and Anton Ghon,[39] is often quite correct if one considers only the findings in infants after death. It frequently happens in fact that when one or more caseous tubercles exist in the bronchial gland group, one or more other tubercles are to be found in the corresponding lung zone. Parrot, Hutinel, and G. Kuss hold these lung tubercles to be the "*chancres d'inoculation*" or primary foci of infection, but there is nothing to prove that they really mark the portal of entry of the bacillus into the body. The fact that many extremely carefully made autopsies reveal no pulmonary tubercle (Marfan, Weigert, Biedert, Bollinger, O. Muller, and others) and, on the other hand, the customary absence of any lung lesion in the tracheo-bronchial adenopathy of animals killed three to four weeks after experimental infection by the instillation of cultures or bacillus-containing sputa upon the healthy mucous membrane of the eye (Calmette),—while, if one waits longer, isolated or solitary tubercles identical with what

[37] Compt. rend. Soc. de biol., 1876, **28**, 308.

[38] *L'hérédité parisitaire de la Tuberculose humaine.* Paris, 1898, Asselin & Houzeau.

[39] *Primäre Lungenherd bei der Tuberkulose der Kinder.* Berl., 1912, Urban & Schwanzenberg.

Parrot calls the primary foci of infection, appear on the surface of the lung,—prove that these latter are but secondary manifestations of the so-called primary tuberculosis of the hilic glands.

It can be easily demonstrated that in cases of spontaneous infection, even though massive, no *local* lesion is produced as a rule at the point of penetration of the bacillus. The supposed "chancre of inoculation" therefore implies in no way that the virulent elements from which it develops were deposited by the inspired air at the very place of its appearance. To be convinced one need only repeat the following experiment which I performed with V. Grysez.[40]

Upon the mucous membrane of one of the eyes, in a series of guinea pigs, we instilled one drop of a suspension containing 0.5 mgm. of a culture of bovine bacilli in physiological salt solution. The animals were killed successively after 2, 4, 6, 8, 12, 15 and 18 days and from each one there were removed, separately and aseptically: the retro-mastoid, retro-pharyngeal and cervical glands, the tracheo-bronchial glands and the lungs, spleen and liver. The organs thus removed were completely ground up in physiological salt solution and inoculated into guinea pigs, all of which were sacrificed at the end of three months, none having succumbed in the interval.

The results showed that bacilli were present in the lungs as early as the fourth day and that by the sixth day they could, by experimental inoculation, be demonstrated to have been present in the lungs, cervical glands and spleen, before any visible tuberculous lesion was to be made out.

On allowing a longer interval to elapse before killing the animals infected by ocular instillation, it is found that at the end of three weeks, only the glands of the neck are swollen and that frequently a few tubercles already exist in the lungs; later other organs become involved, particularly the spleen and the tracheo-bronchial nodes; then those of the hilus of the liver and the mesenteric nodes.

It seems evident, therefore, that *in the case of a local infection*, ocular, pulmonary, intestinal, cutaneous, etc., *the lymphatic and blood infection becomes general before manifesting itself in the form of miliary lesions in the glands near the site of penetration of the bacillus.*

According to a statement which O. Medin was kind enough to send to me, there were in the Stockholm hospital, from 1842 to 1910 inclu-

[40] Compt. rend. Acad. des sci., 1913, **157**, 981.

sive, among 7590 infants dying in the first year, 622 who were infected with tuberculosis, although dying of the most varied diseases. Examination of the autopsy protocols of these 622 cases showed that there were 194 cases with tuberculosis localized exclusively in the lungs and bronchial glands, but in only 17 cases were the tubercles limited to the lungs alone.

Of 287 children, Medin judged that the primary infection was undoubtedly air-borne and, from his other findings, he concluded that a similar origin was to be ascribed to 97.7 per cent of cases of tuberculosis in infancy. This estimate of the celebrated Swedish clinician is approved by many pediatricians. I do not believe it accurate because it takes account only of the results of autopsies which, as I said before, permit one only exceptionally to identify the true portal of entry of the tuberculous virus, and because it discounts all the experimental results going to prove that a lymphatic infection, through any mucous membrane whatever,—that of the eye for example, or of the pharynx, or the digestive tract,—manifests itself usually by glandular lesions to begin with, and by pulmonary lesions afterward, in circumstances where aerogenous infection is out of the question.

It cannot be denied that the tuberculous virus may enter into the body by the pulmonary route; but, for the reasons which I have stated above, penetration by this path is certainly much more difficult than through the mucous membranes of organs which are directly exposed to air contamination or through *those whose essential function is absorption* (the buccal cavity and the digestive tract). The frequency of primary air-borne infection of the lungs has been much exaggerated, and such is the case because Cohnheim's law imposed itself as a dogma on the minds of physicians up to the actual moment when there was introduced into science the fruitful idea of *occult infections by the tubercle bacillus* (*typho-bacilloses and infections without tubercle formation*).

CHAPTER X

TUBERCULOUS INFECTION BY ABSORPTION FROM THE DIGESTIVE TRACT

A. THE MECHANISM OF DIGESTIVE ABSORPTION OF TUBERCLE BACILLI. THEIR MIGRATIONS IN THE BODY

In the lower animals, such as the fresh water *hydra*, which possess a *digestive sac*, the cells of the entoderm send forth into the interior of the cavity pseudopods which are like those of the *amebae* and which ingest solid particles capable of serving as food.

In the higher animals, the enormous absorbing surface of the digestive tube is covered almost throughout with fixed epithelial cells which do not possess the same properties.

The stratified pavement epithelium which covers the esophagus, the dense layer of cylindrical, prismatic or pyramidal cells which line the stomach, the great number of glands which empty their abundant secretions upon the surface of these structures, as also the very nature of the secretions, all of these elements do not *normally* permit the passage of leucocytes through their walls. These migrations can take place only in consequence of some sort of an irritation lesion or through a traumatism.

Moreover, the so-called primary tuberculous localizations occur there only very exceptionally. Tuberculous lesions of the esophagus and stomach in man, and of the true stomach and rumen in cattle are cited as pathological curiosities.

On the other hand, the phenomena of absorption are accomplished with increasing intensity from the duodenum to the termination of the small intestine, and with diminishing intensity from the ileocaecal valve to the rectum. They do not consist solely in a simple penetration by osmosis of the food substances dissolved by the digestive juices. It is known today that the intestinal epithelium permits the transit of a variety of protein substances and fats previously split into fatty acids and glycerins. It is also known,—and this fact is all important for the question which concerns us,—that bacterial

bodies and certain mineral particles in fine suspension in the chyme are constantly transported during digestion from the interior of the intestine into the central chyle-bearing vessels of the villi.

This passage outward is accomplished through the intermediary of the *wandering cells* which penetrate *between the cylindrical epithelial cells of the intestinal epithelium* or, as Renault puts it, *into the very interior of the latter* which they transform into true *fenestrated cells* (*temporary stomata*). It is specially active at the level of *Peyer's patches* which represent in a certain sense veritable *lymphatic sponges* and which are particularly abundant in the ileum.

In order to understand the mechanism of absorption of bacteria from the intestine, it should be recalled that the food mass, which contains the microorganisms in relatively large number, does not pass through the digestive tract as though gently pushed along in the interior of a cylindrical tube of uniform diameter. It progresses very slowly, and intermittently, under the control of peristaltic and antiperistaltic movements which cause it to penetrate into one after another of the deep pocket-like depressions between the valvulae conniventes and between the villi.

If one admits with Sappey that the small intestine alone has a capacity of 6 to 8.8 liters and a surface area of 10.125 square meters, not including that of the villi, one can form an idea of the very favorable conditions there existing for the introduction of infectious elements into the lymphatic circulation of which the highly developed superficial network collects all the chyle produced during digestion.

When a bacterium (tubercle bacillus or other variety), conveyed by a wandering leucocyte, has penetrated into a chyle-bearing vessel, it follows the current of the lymph which floats it first *through the filtering lymph nodes* (*Schaltdrüsen*) and then *into the cavernous sinuses of the mesenteric gland corresponding to the region whence it came.*

The glands of the mesentery, numbering from 130 to 150 in man (Quain), vary greatly in size, from the volume of a millet seed to that of an olive. They are larger in children than in the aged. In cattle they form an almost unbroken beaded chain of flattened drawn-out strands, between the layers of the peritoneum, throughout the length of the suspensory ligament of the intestine.

In these nodes the lymph current is considerably retarded by numerous obstacles opposed to it by the gland sinuses and the connective tissue partitions which separate them. If the bacilli conveyed

are in large number (after a massive infection for example), or if they have had time to multiply in the wandering cells which have phagocytized them and to kill these cells, endothelial reactions, leading to the formation *in loco* of giant cells, immediately supervene and tuberculosis of the gland will make its appearance.

A little later, as caseous softening occurs in the earliest tubercles, the infection will by degrees involve other gland groups along the course of the efferent lymphatics, or it will set up a general infection of the body by disseminating a greater or lesser number of bacterial elements into the lymph stream, the latter in man being poured into the blood mass by the thoracic duct at its confluence with the left subclavian vein.

But if it happens that the infecting bacilli are isolated, few in number, or not very virulent, the leucocytes ingesting them remain unharmed, despite the presence of these undigested parasites in the cell protoplasm; they preserve their motility and continue their migrations in the lymph or blood circulation of the various organs up to the time when, sooner or later, they end by undergoing death. Then, at the point where the dead cells form a capillary embolus, perhaps far removed from an obscure portal of entry of the bacilli a tuberculous lesion develops. Thus, following a non-massive infection, of no matter what origin, whether produced through an excoriation of the skin, through a healthy mucous membrane, by way of the respiratory passages or by the intestine, there appears occasionally an isolated localization of tubercle bacilli in some organ, be it lung, pleura, joint or other serous membrane, bone, testicle or ovary, larynx, etc. But the lung is the most liable to be the seat of this localization, by reason of the immense surface which it presents for the development of a blood and lymph capillary system which are here more extensive and delicate than anywhere else. *The great frequency of so-called primary tuberculosis of the lung is therefore due to the fact that it represents the first manifestation of a bacillary infection, which may have occurred by way of any lymphatic or blood route, often long before the appearance of disease, and which may have remained latent possibly for years.*

If the reader will keep well in mind what has already been said, he will now be convinced without difficulty of the fact that most tuberculosis, in earliest infancy as well as in adult life, has its origin in intestinal infection, at times very remote or again very recent, massive or

discrete, more virulent or less virulent, and which leaves no trace in the glands adjacent to the portal of entry (Law of Cohnheim) except when the number and character of simultaneously penetrating bacilli oblige the body to immediately expel them by setting its defensive cellular reactions at work. The latter event takes place, as we shall see later, only in subjects already previously infected and rendered partially *immune* (*phenomenon of* Koch).

B. EXPERIMENTAL DEMONSTRATION OF THE PASSAGE OF TUBERCLE BACILLI THROUGH NORMAL DIGESTIVE MUCOUS MEMBRANE.—THE COURSE WHICH THEY FOLLOW IN INFECTING THE LUNGS OR OTHER ORGANS

Long before any experimental research had been undertaken as to the transmissibility of tuberculosis by the ingestion of infectious material, Malin[1] had published the very interesting observation of two dogs belonging to a phthisical woman of 58 years. Both dogs had greedily swallowed her sputa and had died one after the other with enormous suppurative lesions of the two lungs.

But without doubt we owe the demonstration of transmissibility of tuberculosis by the digestive tract to the excellent experiments carried out and published by Chauveau from 1868 to 1872.[2]

This scientist furnished the first examples of tuberculosis of the lungs and of the bronchial and mediastinal glands, of certain intestinal origin, *in which no trace of lesions at the portal of entry of the virus could be detected.*

It should be acknowledged also that we are indebted to him for the conception,—which today appears so important,—that, following the ingestion of tuberculous material, primary pulmonary tuberculosis, with or without tracheo-bronchial adenopathy, may occur in calves, regardless of whether the infecting virus be of human or bovine origin.

From 1868 Chauveau was writing, "it is evident that the natural and spontaneous contagiousness of tuberculosis cannot be attributed exclusively to infection of the surrounding atmosphere by air expelled from the lungs of phthisical subjects. Animals confined in the same

[1] Gaz. médicale de Paris, 1839, p. 634.

[2] Bull. Acad. méd., 1868, **33**, 1007; (Letter to Villemin) Gaz. hebdomadaire, 1872, 215; Assoc. pour l'avanc. des sci., Lyon, 1873, p. 727; Lille, 1874, p. 943; Compt. rend. Acad. des sci., 1907, **144**, 777; 817.

stable or in the same pastures, drinking from a common spring, tank or receptacle, have there the opportunity to be constantly swallowing mucous discharges from the noses of other animals. Now, if these excretions are from phthisical animals, they cause tuberculous infection. This is likewise true for the human race."

And the following conclusion, the truth of which becomes more obvious every day, stands out from these researches:

The digestive tract, in man as in cattle, constitutes one path of tuberculosis infection, and it may indeed come into play more frequently than the pulmonary.

By a long series of experiments first at Hanover and then at Berlin, Gerlach[3] confirmed in 1870 the facts announced by Chauveau and was the first to demonstrate that tuberculosis could be transmitted to healthy animals by the ingestion of milk from tuberculous cows.

Meanwhile Villemin,[4] Parrot,[5] and a little later Klebs, Gunther and Harns, Saint-Cyr, Viseur,[6] Toussaint,[7] Peuch,[8] Baumgarten, Wesener, Perroncito, Sydney Martin, Schottelius, Nocard and Rossignol, de Haan, Vallée,[9] and others published a large number of facts proving that the rabbit, guinea pig, cat, dog, pig, sheep, goat, ox, and monkey contract tuberculosis following the ingestion of various tuberculous products (milk, sputa, ground-up organs); that these different animals show a variable species susceptibility, being greater in ruminants than in carnivores; and finally that infection is more easily produced in the young than in the old.

And yet, in many cases, attempts at infection were unsuccessful, even though repeated, and the reason could not be discovered. (Colin, Semmer). Straus and Wurtz[10] supposed that the gastric juice intervened to destroy the bacilli, but experiment proved to them that this was not the case. At that time the great differences in the virulence of tuberculous material, according as it was of bovine or human origin, were not understood. It was supposed therefore that the resistance offered in certain cases to penetration of the bacilli

[3] Jahresb. der Tierarztl., 1869, p. 6.
[4] Gaz. hebdomadaire, 1869, p. 260.
[5] Soc. méd. des hôp., 1869, March 12.
[6] Bull. Acad. méd., 1874, **38**, 890.
[7] Compt. rend. Acad. des sci., 1880, **20**, 754.
[8] Ibid., 1880, **20**, 1581.
[9] Internat. Congr. on Tuberculosis, Paris, 1905.
[10] Arch. de méd. expér., 1889, **1**, 370.

through the digestive tract was due to the protection of the epithelial covering of the mucous membrane although Chauveau, Wesener,[11] and later Dobroklowski,[12] had already insisted upon the ease with which the tuberculous virus could pass through the *normal* epithelial lining of the intestine, without producing any apparent lesion.

But this idea was meeting with opposition. Robert Koch, and Baumgarten believed that the bacillus always left its trace in the form of a visible lesion at the point of penetration.

Meanwhile Desoubry and Porcher,[13] in Nocard's laboratory at Alfort, had established the fact that many bacteria of all sorts pass through the intestinal mucosa during the digestion of fatty substances and are found for several hours in the chyle and in the blood. This finding has been verified so often since then that it is now a rule in all institutes of serotherapy to bleed the horses only when fasting, if sterile sera are to be obtained.

Furthermore, Nicolas and Dercas[14] demonstrated the same fact as regards the tubercle bacillus. They incorporated virulent cultures into a fatty soup which was fed to dogs. When these animals were killed at the height of digestion, 3 hours after the infecting meal, and chyle was collected from the *receptaculum chyli* and inoculated into guinea pigs in doses of 5 to 10 cc. tubercle bacilli were found with doses which were very small in comparison with the whole amount of fluid absorbed by the chyle-bearing vessels during the three hour interval.

Maz. Ravenel, Von Behring and Roemer, Bisanti and Panisset,[15] Ficker, Oberwarth and Lydia Rabinowitsch[16] repeated these experiments and found that after a meal of infectious material, not only the lymph but also the heart-blood frequently contained bacilli, and that the results were the more constant the younger the experimental animals employed, the intestinal wall of suckling animals permitting the passage with the greatest facility.

In the new-born in fact,—as Disse[17] has shown (and this is a fact

[11] Habilitationschrift, Freiburg, 1885.

[12] Arch. de méd. expér., 1890, **2**, 253.

[13] Compt. rend. Soc. de biol., 1895, **47**, 101.

[14] Ibid., 1902, **54**, 987.

[15] Compt. rend. Soc. de biol., 1905, **58**, 91.

[16] Berl. klin. Wchnschr., 1908, **45**, 298.

[17] Ibid., 1903, **40**, 4.

on which V. Behring rightly insisted in support of his theory as to the childhood origin of pulmonary tuberculosis in adults), the epithelial cells of the intestine are entirely protoplasmic; the true mucous membrane does not appear until some days after birth. The result is that the intestine, during the first weeks of life, is permeable not only to bacilli but also to albuminoid substances, to toxins and to antitoxins.

The researches which I carried out with C. Guérin,[18] later confirmed by Martin Hermann (of Mons),[19] then by A. S. Griffith[20] before the *English Royal Commission*, enabled us from the very beginning to institute a method which is always successful in artificially infecting animals by the digestive tract. The method consists in having the tuberculous virus mixed and taken in with the food, the bacilli (thanks to a sufficiently prolonged grinding in an agate mortar with a little yolk of egg or beef bile) being so divided that they remain finely emulsified as in milk or sputum. Under these conditions a single infecting meal is sufficient to assure the absorption of a certain number of bacilli and to produce tuberculous lesions which, in young animals, remain quite often in the mesenteric glands, but which, in adults, appear most often first in the lungs.

By autopsying the animals (guinea pigs, goats, cattle) at longer and longer intervals after the single infecting meal, we could follow the course which the bacilli pursue to reach the lungs. They pass through the intestinal mucous membrane, as shown by Chauveau and then Dobroklowski, *without leaving the slightest trace of their passage* and, when arrived in the chyle-bearing vessels of the villi, are already found to be the prey of the leucocytes, as I was able to satisfy myself with Vansteenberghe.[21] The leucocytes bear them along in turn as far as the nearest mesenteric glands.

In suckling animals, particularly in massive infections, these glands retain the bacilli, since these organs during early life, as Weigert[22] had shown, serve as an almost perfect filter for the lymph. Now and then bacilli are destroyed in the course of time or modified to the point of being harmless (J. Bartel), again they initiate tuberculous

[18] Ann. de l'Inst. Pasteur, 1905, **19**, 601; 1906, **20**, 353.
[19] Bull. Acad. roy. de méd. de Belg.; 1908, 4. s., **22**, 739.
[20] 2nd. Interim Rep. Roy. Comm., Appendix, iii, 219.
[21] Ann. de l'Inst. Pasteur, 1910, **24**, 316.
[22] Deutsch. med. Wchnschr., 1903, **29**, 735.

lesions which, going on to caseation, discharge their bacilli into efferent lymphatic channels and into the blood circulation.

In older animals, whose lymphatic glands have a looser structure (widened sinuses, stretched connective tissue framework) and are much more permeable, the bacilli—always phagocytized in polymorphonuclear leucocytes—are borne along with the lymph from the thoracic duct as far as the right ventricle of the heart and thence sent into the capillaries of the lung. If, as I have said above, the bacillus-containing leucocytes have already lost their ameboid movements, they are incapable of passing by diapedesis through the walls of these capillaries and they there form minute emboli which become the site of so many tuberculous formations at the expense of the vascular endothelial walls (gray granulations).

Schlossmann and Engel[23] mixed finely separated bacilli in milk or in cream and introduced the emulsion directly into the stomach of young guinea pigs through incision of the abdominal wall. They then killed the animals at varying intervals and found that inoculation of their lungs into other guinea pigs proved virulent as early as after six hours. Orth and Lydia Rabinowitsch[24] performed similar experiments with dogs and swine with practically the same results.

Therefore it must be admitted that tubercle bacilli pass very rapidly through the healthy intestinal wall, especially during the digestion of fats, and that they cause no lesion of the mucous membrane nor leave behind the slightest trace at the point of penetration. And these bacteria, poured with the lymph into the blood circulation, may, according to circumstances (number of infectious elements absorbed, degree of virulence), lead to an occult purely glandular infection, or give rise from the first to multiple grave lesions tending to localize in the lung by reason of the peculiar anatomical structure of that organ.

If some clinicians still find this truth so difficult to accept, it is because Cohnheim's *law* weighs upon them as an intangible dogma and they are forever preoccupied during their autopsies with searching for evidence of the virus at its portal of entry into the body. Indeed, as I have already stated and many experimenters have shown, *this law holds only in massive infections*, such as are ordinarily brought about in experiments with alimentary infection, inhalation or sub-

[23] Deutsch. med. Wchnschr., 1906, **32**, 1070.
[24] Virchow's Archiv., 1907, **190**, Beih., 1.

cutaneous inoculation in animals. It stands out as applicable also in certain observations; for example in those of Demme bearing upon four children who died during the first year with intestinal tuberculosis. They had been infected by a phthisical nurse who contaminated their food by trying its temperature with her lips. It applies also to certain other cases reported by Oscar Wyss, by Kossel, Zinn, Gruneberg, Baumgarten and by G. Küss in his admirable work on "*l'hérédité parasitaire de la tuberculose humaine.*"[25] Lastly it applies *in subjects already infected.*

But in all the cases which are revealed only by the tuberculin reaction, and which are today recognized as so numerous, the *law* of Cohnheim does not hold. It is not valid in animal experimentation. Chauveau, Haan, Von Behring and Roemer, Weleminsky, Arloing, and Vallée, have furnished abundant proof of this, as I myself have also done with C. Guérin. Nor does it hold in a number of clinical observations made with the greatest care. The case reported by Roger and Garnier[26] and that by M. Letulle[27] are particularly illuminating in this respect.

Roger and Garnier reported the case of a woman affected with miliary tuberculosis and who died 17 days after her accouchement, without there having been any clinically detectable tuberculous lesions of the mammary glands. This woman nursed her baby during only two days. The latter died six weeks after birth, 12 days later than the first guinea pig which was inoculated subcutaneously with the milk of the mother. The child showed miliary tubercles in the mesenteric glands, liver, spleen, and kidneys. There was no visible intestinal lesion on direct examination, nor was any to be found with the microscope.

Letulle's case was one of meningeal miliary tuberculosis secondary to multiple subacute tuberculous tracheo-bronchial glands, apparently primary; and an old chronic fibrous adenitis of the intra-mesenteric glands, serving to reveal the path of entry of a tuberculous infection contracted in childhood. The author's conclusions are the following:

"The patient, as a child, suffered from a tuberculous infection of some of the mesenteric glands, without any alteration of the intestine being produced by the bacilli.

[25] Asselin and Houzeau, Paris, 1898.

[26] Compt. rend. Soc. de biol., 1900, **52**, 175.

[27] Bull. mém. Soc. méd. d. hôp. de Par., 1907, 3. s., **24**, 709.

"The resulting chronic mesenteric adenitis remained benign and walled off and was able to reabsorb the bacillary products.

"It allowed to pass, or conducted through itself, a few bacilli which reached the mediastinal glands, particularly the peri- and sub-tracheal and peribronchial groups, and there, these bacilli multiplied extensively.

"While the mesenteric adenitis was becoming thoroughly cicatrized, the caseated glands of the mediastinum, far from encapsulating their virulent products, continued to progress until the day when they reached the point of discharging the ultra-poisonous culture into the circulating blood stream. The result was the secondary infection of the meninges, lungs and liver (in the interior of which I succeeded in finding a few scattered quite recent miliary tubercles, barely beginning to caseate), the terminal step in the process.

"Thus," adds Letulle, "this remarkable case shows, in my opinion, the complete cycle of a miliary tuberculosis orginating long before in the intestine, without lesions of the digestive mucous membrane, but which, before its meningeal phase, passed through two successive glandular stages; a first or mesenteric stage which succeeded in cicatrizing itself completely, and a second or mediastinal stage which, on the contrary, offered a very favorable soil for the progressive multiplication of the tubercle bacillus and for the increase of its virulence."

H. Beitzke,[28] during 1907 and 1908, examined with the utmost care 1100 subjects which he autopsied. The examination was made organ by organ, many being restudied on several occasions. When no organs showed tuberculosis, he proceeded to the examination group by group of the lymph nodes of the digestive tract, and to the examination of the intestine. All glands suspected of tuberculosis, particularly those which were calcified, and all the suspicious points of the intestine were included and examined under the microscope. Half of the glands were inoculated into rabbits, the calcified parts having been previously triturated in sterile mortars.

Proceeding thus, Beitzke found 13 cases of primary tuberculosis of the intestine (8 children and 5 adults). The inoculation results were in agreement with the microscopic in only 2 out of 10 cases. In 3 cases microscopic examination was negative where inoculation was positive.

[28] Virchow's Arch., 1907, **190**, Beih., 58.

Among the 1100 autopsies, primary tuberculosis of the intestine occurred in 4.4 per cent. There were 397 children of whom 49 were unquestionably tuberculous. Among the latter were 8 cases of primary intestinal tuberculosis, that is 2 per cent and 16.3 per cent of the totals.

These results agree with those of Lubarsch who examined 1087 subjects.

Beitzke considers that the majority of cases of primary intestinal tuberculosis result from drinking infected milk. Engorgement of the lymphatic glands or retention of bacilli in the latter may, in his opinion, serve to reveal the course followed by the virus.

I would recall finally that in collaboration with C. Guérin and Deléarde[29] I was able to show that tubercle bacilli very often exist in apparently absolutely healthy mesenteric glands of children affected with tracheo-bronchial adenopathies, and that we have, as has also Vallée, Weleminski, Orth, and others, made numerous analogous observations in cattle infected through living with others, and in goats and guinea pigs artificially infected through ingestion.

Clinical evidence that the tuberculous virus passes through the healthy intestinal mucous membrane without local reaction is evidently confirmed therefore from the experimental side.

The normal process of infection by the digestive tract, when resulting in tuberculization of the lung, tracheo-bronchial nodes or other organs, is accomplished with a degree of delay which is in contrast with the rapidity of evolution of massive experimental infections by way of the respiratory passages or by subcutaneous inoculation.

The objection raised by Chaussé,[30] in accord with Flügge and his pupil Findel, that the number of bacilli necessary to produce this tuberculization of the lungs is much larger (six million times according to these authors) than the number sufficient to infect by inhalation (50 bacilli acording to Flügge, a single bacillus according to Chaussé) cannot be sustained. Indeed there is no proof that a single very virulent bacillus freshly derived from a lesion in evolution, absorbed with the chyle from the intestine and carried successively into the lymph and blood circulation, is not in itself sufficient to later create a

[29] Compt. rend. Acad. des. sci., 1906, **142,** 1136.
[30] Ann. de l'Inst. Pasteur, 1911, **25,** 518.

tuberculous lesion in the lung or in any other organ. Very fortunately, all bacteria ingested (as well as all those inhaled) are not absorbed. Only a very small number of them pass through the intestinal mucous membrane. The others are expelled with the excrement. But that this penetration occurs can no longer be doubted and we know today, since the work of Schottmüller and of many others, to say nothing of that of my pupils and myself, that not only the tubercle bacillus but also *B. typhosus*, the paratyphoid bacilli, the pneumococcus, the staphylococcus and the virus of epidemic poliomyelitis enter into the blood by the intestinal path.

The history of *glanders*, so closely related to tuberculosis, provides very valuable information, familiar to veterinarians, but too often forgotten by physicians. It is a disease characterized essentially by the appearance of small pulmonary tubercles which are translucent in the beginning and which go on to caseation like tubercles due to the tubercle bacillus. Now the transmission of pulmonary tuberculous glanders to the horse, and even to the ass which is particularly susceptible, is impossible either by cutaneous or subcutaneous inoculation, or even by introducing the virus directly into the trachea or by flooding the pulmonary alveoli. By the cutaneous and intratracheal route the lesions of *farcy* and a *glanders pneumonia* without tubercles are produced. *If however*, as Nocard has shown, *the horse is made to ingest a small quantity of glanders bacilli mixed with the water which he drinks, the lesions of pulmonary glanders with tubercles are produced unfailingly.*

It cannot be denied then that pulmonary tuberculous glanders is always of intestinal origin.

The many experimental facts already cited attest that, as regards tuberculosis, all portals of entry through which the bacilli may pass into the lymphatic or into the blood circulation permit of the invasion of the lung. But they prove further, as Von Behring[31] stated, and as I myself affirmed with Ravenel,[32] Aufrecht,[33] Klebs and many other investigators, *that in all susceptible animals, man included, tuberculosis, in all its various localizations, glandular, pulmonary, etc., particularly in its slowly evolutive forms, results in an immense majority of cases, from an infection which is primarily lymphatic and later of the blood.*

[31] Deutsch. med. Wchnschr., 1903, **29**, 689; 1904, **30**, 193.

[32] Lancet, 1901, ii, 349; 443.

[33] Deutsch. Arch. f. klin. Med., 1903, **75**, 193.

and which originates in the absorption of tubercle bacilli from the digestive tract, principally through the buccal, pharyngeal and intestinal mucous membranes.

This conception dominates the whole history of natural infection. Veterinarians, with rare exceptions, do not contradict it; almost all of them have long been convinced that, in stables, healthy cows infect themselves by swallowing, at the common trough, the secretions of diseased cows or food contaminated by their dejections; that calves, pigs, cats and dogs contract tuberculosis when they are nourished with milk containing the bacilli; and that at times as in the menageries, lions, tigers and other carnivores become infected by eating diseased meat.

Why then are so many physicians unwilling to consider these experimental proofs? They are no longer without the knowledge that their clinical observations and their autopsies,—in the immense number of cases where the *law* of Cohnheim does not fit and where the tuberculosis, *latent* from the beginning and over a long period, has stealthily established itself in the body,—permit them only very exceptionally, or almost never, to discover the initial point of penetration of the virus. Why should they require that man comport himself differently from animals as regards tuberculous infection?

Simply perhaps because the *pulmonary localizations*, common as they are,—and we know the physiological reasons,—are called most frequently to their attention. Probably also from the fact that, before we possessed our newer knowledge of the relative specificity of human and bovine tuberculosis, and when the milk of tuberculous cows was accorded a degree of infectiousness for man which it possesses to only a mild degree, there was an inclination to regard *intestinal origin* and *alimentary origin* as identical.

But now that we are more enlightened, this confusion is no longer excusable. And it must be stated, because true, first, that *for man, the principal factor in infection is the bacillus freshly derived from tuberculous man*, and secondly that *the path of digestive absorption is one of those which are open to the exterior and which offers itself most frequently and most readily to the penetration of the virus into the body.*

CHAPTER XI

FREQUENCY AND PATHOLOGICO-ANATOMIC CHARACTERISTICS OF TUBERCULOUS INFECTION IN CHILDREN

The young individual, entirely free from tuberculosis, is extremely susceptible to infection by the tubercle bacillus. The more recently the strain is derived from a diseased body of the same animal species, the higher the virulence and the more rapidly progressive and fatal are the lesions produced. Contributing to the virulence of the infection are the age of the host, the size of the dose and the frequency with which the bacilli are introduced.

As I have already said, we are particularly indebted to the work of Landouzy[1] (1886–1891), and to that of Baginsky, of Comby, of Hamburger and Sluka, of Emmet Holt, Kuss, Hutinel, and others for our knowledge of the fact that, far from being rare in the first months of life as was previously thought, tuberculosis is so frequent in very young infants that it is encountered in about one-third of the autopsies of babies under two years.

In the course of the first year, according to Landouzy, 27.8 per cent and, during the second year, 16.2 per cent of deaths are due to this disease.

According to H. Barbier and Bondon, out of each 1000 children from birth to 15 years 392 die, and of these 116, or 29.2 per cent succumb to tuberculosis. This mortality is apportioned as follows:

AGE	TOTAL MORTALITY		ACTUAL MINIMUM MORTALITY FROM TUBERCULOSIS	REMAINING ALIVE AT THE END OF THE YEAR
	Per cent	Number of children		
From birth to 1 year	20.0	200	30 (to 60)	800
1 to 2 years	6.5	48	12	752
2 to 3 years	3.5	26	11	726
3 to 4 years	2.3	16	7	710
4 to 5 years	1.6	12	7	698
5 to 10 years	8.4	56	33 (in 5 years, or 6.5 per year)	642
10 to 15 years	5.5	34	16 (in 6 years, or 2.6 per year)	608

[1] Rev. de méd., 1887, 7, 383.

These figures should be regarded as approximately accurate, but if anything rather below the actual state of affairs, since we know that many tuberculous lesions are not found, even though sought for with the utmost care at autopsy. And further it must be remembered that a very large number of children who die outside of hospitals without the diagnosis being established by autopsy, have in reality died as a result of tuberculous infection.

It is likewise a fact well known among clinicians that in the infant, particularly up to the age of 6 months, tuberculosis is almost always of extremely grave prognosis. Unfortunately it intervenes also with great frequency as a fatal complication at the end of other illnesses which in themselves are not serious.

I shall borrow from an article by Léon Bernard[2] the following table which summarizes the principal statistics published in France and in Germany:

Percentage of childhood mortality from tuberculosis

	HUTINEL	KUSS	COMBY	MANTOUX	KOSSEL	HAMBURGER	BINSWANGER
From birth to 3 months		1.16	2		1.6	6	2.2
From 3 to 6 months	3.5	13	18	7	11	17	8.4
From 6 to 12 months	—		27	16		22	16.8
Second year..................	33	24	43	23			
2 to 4 years..................		50				30	
Puberty......................						53	

A. TUBERCULOSIS OF THE LUNGS AND TRACHEO-BRONCHIAL GLANDS (TUBERCULOSE GANGLIO-PULMONAIRE)

Following upon, and generally as a consequence of tubercle bacillus septicemia, which has already been discussed in a preceding chapter (VII), the organs particularly involved in tuberculous infection of infants are, first, *the lymphatic glands*, especially the tracheo-bronchial and mediastinal groups (*tuberculosis of the lung hilum*), the mesenteric groups, and the lung, spleen, liver, meninges, the brain and the serous cavities (pericardium, peritoneum, pleura).

Among 816 tuberculous children at the Sanatorium of Belzig, W. Freymuth[3] found pulmonary lesions in but 10 per cent whereas

[2] Tuberculosis, July 1908, Presse médicale, April 18, 1914.
[3] Beitr. z. klin. d. Tuberkulose, 1912, **23**, 135; 532.

PLATE VI

1. Glandular-pulmonary tuberculosis, primary infection in an infant of 6 months. Considerable tumefaction of the packet of tracheo-bronchial glands. A few isolated tubercles in both lungs.

2. Caseous pulmonary tuberculosis of childhood with adherent pleura (massive primary infection).

3. Caseous pulmonary tuberculosis of childhood. Cavities and disseminated cheesy food (massive primary infection).

(From anatomical specimens in the collection of Professor Deléarde at Lille.)


I
II
III

90 per cent had glandular lesions; but these figures are based simply on clinical signs and not upon autopsy findings. In 80 per cent of the children there was no doubt as to family infection.

In 119 autopsies of tuberculous children, Emmet Holt[4] of New York found pulmonary lesions in 99 per cent, pleural in 58 per cent, tracheo-bronchial gland lesions in 96 per cent and lesions of the pericardium in 6 per cent. The last are almost always secondary to tuberculosis of the mediastinal glands.

On the other hand, Hamburger and Sluka[5] (of Vienna), in 160 cases, found pulmonary lesions in only 50 per cent, while the tracheo-bronchial glands were affected in 96 per cent. D'Espine[6] (of Geneva) arrived at the same conclusions. In the opinion of this eminent clinician, the latent form of tuberculosis of the bronchial glands "without accompanying pulmonary lesions" is infinitely more frequent in practice than autopsy results would lead one to suppose.

Of 78 infants dying of acute tuberculosis and of meningitis, Haushalter and Fruhinsholz found the mediastinal glands to be tuberculous in 74 cases.

The statistics of Comby on this point are most important and most suggestive. Among 1515 autopsies performed during 15 years, tuberculous lesions were found 569 times (37.55 per cent) and *in every case there was a tracheo-bronchial adenopathy.* It should be remarked that this figure includes acute tuberculosis as well as other forms progressing more slowly. It would seem then that, in accordance with the law of Buhl, which admits of but few exceptions in children, *acute tuberculosis originates in a focus of tubercle bacilli, most often localized primarily in the lymph nodes of the mediastinum.*

This primary localization is brought about by the numerous anastomoses which unite the mediastinal glands with the supra and sub-diaphragmatic lymphatics and which, to use the expression of Weleminsky, make of them a veritable *lymphatic heart* (*coeur lymphatique*).

Among 47 autopsies of children under one year, made by G. Hedren,[7] 26 subjects presented only bronchial gland and pulmonary lesions,

[4] *Tuberculosis in infancy and childhood*, Lond., 1908, Kolynack.

[5] Jahrbuch d. Kinderheilkunde, 1905, **62**, 517.

[6] Bull. Acad. méd., 1907, **57**, 167.

[7] Ztschr. f. Hyg., 1913, **73**, 273.

with no apparent affection of the mesenteric glands or intestine. In the 21 other subjects there were glandular lesions in various organs other than those of the thorax. Among the 26 subjects of the first group, there was one in whom the tracheo-bronchial glands were caseated without any evidence of a pulmonary focus.

According to Hedren, in unilateral pulmonary tuberculosis, the tracheo-bronchial glands are almost always caseated on both sides. Only four of his autopsied subjects proved exceptions to this rule.

In 11 cases out of 25 there was only a single pulmonary focus, which was almost always sub-pleural. Of these cases 8 were localized to the right side and 3 to the left. The single pulmonary focus was situated 9 times in the lower lobe.

Among the 21 subjects of the second group, 7 presented a primary infection of the cervical glands and the 14 others had pulmonary lesions (8 on the right and 6 on the left), unilateral 9 times and single 6 times.

In all but 3 of these subjects, the mesenteric glands were more or less caseated; 8 had tuberculous ulcerations in the small intestine, 1 had them in the large intestine and 9 in both the small and the large intestine.

Summing up the observations relative to his 47 autopsies on tuberculous infants under one year, Hedren gives the following table which indicates the relative frequency with which each separate organ is involved in the tuberculous infection of nurslings:

	per cent
Bronchial glands	100
Lungs	97.8
Spleen	82.9
Liver	61.7
Mesenteric glands	57.4
Intestine	38.3
Meninges and brain	36.6
Kidneys	34.0
Cervical glands	22.9
Heart	10.6
Pancreas	4.2
Suprarenal capsules	2.1
Tonsils	2.1

At about the same time as Hedren, Anton Ghon[8] published a memoir in which he reached practically the same conclusions. The autopsy studies of this author were upon 184 subjects.

It can be said therefore that as a general rule, *tuberculosis of infants without tracheo-bronchial adenopathy does not occur.* The glandular disturbance may assume different forms according to the glands chiefly involved. These glands, as Guéneau de Mussy and Barety have shown, are divided into 4 large groups:

1. The *right pre-tracheo-bronchial*, or *right juxta-tracheal* group, in the angle formed by the trachea and the right bronchus, in relation anteriorly with the superior vena cava and arch of the aorta; posteriorly with the right pneumogastric; on the right with the superior lobe of the right lung; on the left with the trachea; below with the right and left branches of the pulmonary artery; above with the subclavian artery and recurrent laryngeal nerve. This is the most important group.

2. The *left pre-tracheo-bronchial*, or *left juxta-tracheal group*, in relation above with the aorta and recurrent laryngeal nerve; below with the root of the left lung.

3. The *inter-tracheo-bronchial* group, underneath the bifurcation of the trachea, in relation below with the pulmonary veins; posteriorly with the esophagus, the aorta and the azygos vein.

4. The *peri-bronchial* groups, formed by the extremely numerous and for the most part very small glands (lymphatic follicles) which accompany the bronchi and their ramifications throughout the mass of lung substance.

All of these glands communicate freely among themselves and also with the lymphatics of the trachea, bronchi, lungs and pleura, with the small sub-pleural glands, with the cervical gland chains and with the sub- and supra-diaphragmatic and retro-sternal glands. The lymph passing through them ebbs and flows like a tide, back and forth between center and periphery. Their whole principal mass,—to repeat the simile of Welcminsky,—is like a *lymphatic heart,* alternately dilated and contracted by the movements of the lungs and the pulsations of the aortic arch.

The largest glands are those which surround the hilum of the lung. In some tuberculous children they attain the size of an apple.

[8] *Der primäre Lungenherd bei der Tuberkulose der Kinder,* Berl., 1912, Urban & Schwarzenberg.

Their appearance depends upon the age of the lesions within them. At times they form a pink mass with yellow softened caseous points, again they take the appearance of a cooked chestnut filled with an almost dry friable cheesy material; or they may resemble a veritable multilobular cyst with a dense fibrous shell, containing creamy grumous pus.

Because of their relations with the structures of the mediastinum, the tuberculous glands may incite serious lesions in their neighborhood; such as compression and ulceration of the trachea and of the bronchi, compression of the superior vena cana and of the pulmonary vessels, tuberculous pericarditis, pressure upon the right pneumogastric, the recurrent laryngeal, and sometimes the phrenic nerve. Finally they may provoke ordinary lesions of the bronchi and lung through mixed infection of which they are often the seat. "Thus," says Hutinel, "are explained the passing bronchitis and temporary congestion in the mild cases; and, in the grave cases, the bronchopneumonia and lesions of pulmonary gangrene observed now and then at autopsy."

Tracheo-bronchial adenopathy, we know today, can no longer be regarded as the result of an infection *primary* in the lung; it is in reality the *initial localization* of a tuberculous process whose source can lie only in lymphatic absorption or in an accidental inoculation. So that the old law of Parrot (*loi des adénopathies similaires* or law of similar adenopathies, according to which, for every finding of caseous bronchial glands at the autopsy of a child there should be revealed one or more tuberculous lesions in that portion of the lung whose lymphatic vessels drain into the affected glands) is valid only on condition that it be inverted. It should be made to state that, in the young child, *consecutive to a tracheo-bronchial gland infection, whether primary or secondary, there appears almost always a more or less discrete or confluent eruption of tubercles in one or several of the innumerable lymph follicles situated in the zones of pulmonary parenchyma which are bathed by lymph sent to them by these glands.*

In children at autopsy as in experimental infections, lesions are frequently encountered in which the upward extension of the infection can be followed, passing for example from the bronchial to the supra-clavicular glands. But as a rule glandular infection follows the descending path more easily.

Experimental proof is clearly furnished by the already stated facts relative to *infection by simple instillation* of sputum or bacillus-containing liquids upon the mucous membrane of the eye of young animals (Calmette and V. Grysez[9]). Following this infection (the reproduction of what occurs when a phthisical mother wipes away the tears of her baby with her handkerchief damp with bacillus-containing-sputum), there appear with mathematical regularity a successive engorgement of the gland chain of the neck and of the tracheobronchial glands. Then, secondary to this last involvement,—in animals infected simultaneously and killed at proper intervals,—one or more tuberculous lesions develop in the lungs.

Loomis[10] thinks that the bacilli can also go from the glands to the lungs by way of the veins.

But whatever the orgin of the pulmonary lesions observed in young infants, it is remarkable to find that the reactions set up about them are often weak or entirely lacking. In the immediate neighborhood of visible tubercles, the lung parenchyma appears scarcely inflamed at all, whereas the glandular reactions are very active.

Unfortunately the child *totally free from tuberculous infection* and on this account highly susceptible to the tubercle bacillus, is, for the same reason, susceptible to other varieties of virulent bacteria which may gain entrance by the same paths. This explains the frequency of the *mixed processes* which contribute largely (if not indispensably as certain authors think) to the production of caseous pneumonias and broncho-pneumonias.

Fairly frequently one observes in the young child, in the absence of these mixed processes and in the presence of a caseation of the packet of tracheo-bronchial glands, the formation of pulmonary cavities surrounded by a more or less thick zone of hepatized tissue. These cavities may attain the size of a large hazel-nut (*see Plate VII*).

In a general way, tuberculous lesions of early life, up to the fourth year,—and the same is true of tuberculosis-free adults of the black race suddenly transported from central Africa into European countries (A. Borrel),—are most frequently the result of a massive infection and but seldom regress. They become rapidly caseous and do not calcify; nor do they undergo fibrous transformation except

[9] Compt. rend. Acad. des sci., 1913, **157**, 981; 1914, **158**, 1315.

[10] Studies from the Loomis Lab., 1890, vol. I; J. Am. Med. Assn., 1891, **16**, 98.

PLATE VII

1. Pulmonary tuberculosis with cavity formation and tracheo-bronchial adenopathy in an infant of 13 months whose mother died of phthisis three months after the child's birth (from Professor Deléarde at Lille).

2. Acute miliary tuberculosis of the lung in the child. Section through the packet of glands (from Professor Deléarde at Lille).

I

II

very rarely. Furthermore they have a most fatal tendency to disseminate themselves and become generalized. Miliary tuberculosis is observed, according to Hamburger and Sluka, in 73 per cent of tuberculous babies while 48.3 per cent according to Still,[11] die of meningitis.

On the other hand, *beginning with the age of 4 years, resistance on the part of the body commences to manifest itself in its disposition to form fibrous tissue and thus to restrict the spread of the tubercles.* Tubercle bacillus infection then tends to assume the characteristics usually presented in the adult.

Now in the latter, *caseated* tracheo-bronchial lymph nodes are almost never found; it is even very seldom that they are found *calcified* (Widerhofer).[12] The caseous softening of these glands therefore almost constantly leads to death in early life.

In the adult, mediastinal adenopathy is none the less common, but it manifests itself only by mild symptoms and remains latent even in phthisical cases.

Herein we have a conception of the greatest importance, to which we shall return later. It enlightens us as to the circumstances which produce a sort of immunity in subjects who have been only accidentally or mildly infected during early life, an immunity of a kind which renders these individuals capable of protecting themselves against reinfection.

B. TUBERCULOSIS OF THE MENINGES (SEE CHAPTER XIV)

The most common form of meningitis in children manifests itself by an eruption of miliary tubercles along the course of the blood vessels and by an intense congestion of the pia mater and arachnoid, with an accumulation of serous exudate, rich in lymphocytes, in the sub-arachnoid space. At times, however, lesions localized in patches (*en plaques*) are found and, more rarely, a pachy-meningitis, the result of an extension from a bone tuberculosis to the dura mater. In such a case the lesion may caseate and extend by degrees along the lymphatics to the pia mater, to the arachnoid and even to the subjacent nervous tissue.

Tuberculosis of the pia mater and of the arachnoid is most often of hematogenous origin and results from the arrival of a large number

[11] *Diseases of Children*, 8th edit. Lond., 1905, Churchill.

[12] Gehradt's Handb. der Kinderkrankeiten, 1878, **3**, H. 2.

of tubercle bacilli in the pia mater vessels. The first focus generally develops either in an arteriole or in one of its capillaries. The next stage is reached through the penetration of the bacillus-containing leucocytes into the lymphatic channels (*perivascular sheaths, sub-and supra-arachnoid spaces*), and their diffusion by this path is proved by the predominance of tuberculous lesions in the larger sub-arachnoid spaces as well as in the principal sulci leading from then.

The nodules locate by preference in the depth of the vascular walls and consist essentially in a thickening of these walls by a mass of cells.

Even in the brain, the cell mass at the beginning of the process may limit itself to the pia mater; later on it may also extend to the neighboring nervous tissue.

Metastatic tuberculosis is most generally found in the regions of the base of the brain at the level of the arteries of the *fissure of Sylvius*, and the lesions are usually bilateral; at times however, but one side is affected.

C. ABDOMINAL TUBERCULOSIS

Tuberculosis of the peritoneum is rare in infants less than one year of age; but it becomes more frequent during the second year and still more so in the course of the third. After four years it is no longer commonly encountered. It assumes at times the *ascitic* form, at times the so-called form "*en plaques*" (*tabes mesenterica*). Primary lesions of the intestine are almost never observed at this age, although the glands of the mesentery are often much swollen and caseated.

In the *ascitic* form the whole peritoneum is covered with miliary tubercles and the peritoneal cavity filled with a fluid which is either transparent or slightly cloudy, with membranous flakes in greater or less abundance. Intestinal adhesions, if they exist, are of loose texture.

In the form "*en plaques*" on the other hand, the adhesions are dense and glue together the intestinal loops so that they can no longer be unfolded. The omentum is thickened, hard, and adherent along the greater curvature of the stomach. The small intestine is usually ulcerated, the lesions being manifestly those of reinfection (*phenomenon of Koch*, see Chapter XXXIX). Their site is marked by a mass of grey tubercles jutting outward under the peritoneum and always grouped about blood vessels (M. Péhu).[13] A small quantity

[13] Arch. de méd. des enfants, 1911, **14**, 24.

of purulent fluid is often found in the peritoneum, forming little encysted pockets. The superificial abdominal veins are swollen and conspicuous (*see Plate VIII*).

Peritoneal tuberculosis in children is frequently accompanied by lesions in the liver and spleen. The liver is then sown with small caseous grey masses, localized particularly about the bile ducts. The capsule overlying is thickened and adherent. The liver tissue itself shows fatty degeneration. The spleen is enlarged, and under its capsule, which is thickened like that of the liver, tubercles more or less disseminated and numerous are found.

D. RELATIVE FREQUENCY OF ABDOMINAL TUBERCULOSIS AND TUBERCULOUS MENINGITIS IN CHILDREN.

John Thomson[14] has compared the frequency of abdominal and meningeal tuberculosis in English children, in special hospitals, with that found in other large centers of Europe and in the United States. The figures which he has collected are as follows:

CITIES	PERCENTAGE OF CASES	
	Abdominal tuberculosis	Meningeal tuberculosis
London	1.8	1.5
Birmingham	1.3	1.4
Sheffield	1.3	0.5
Manchester	2.0	0.7
Edinburgh	3.6	2.0
Glasgow	4.6	2.2
Aberdeen	1.2	0.5
Rome	0.56	2.3
Lyons	0.74	1.05
Berne	0.59	0.8
Budapest	2.0	3.4
Vienna	0.46	2.4
Munich	0.18	1.1
Hagenau (Alsace)	0.10	0.12
Christiania	0.99	1.65
Philadelphia	0.14	0.98
New York	0.42	3.9
Boston	0.40	1.19

[14] British J. of Tuberc., 1907, 1, 250.

PLATE VIII

1. Intestinal tuberculosis in the child (primary infection). (From a specimen at the Bureau of Animal Industry at Washington). Ileo-caecal region with the appendix.

2. Intestinal tuberculosis in the adult. Annular tuberculous ulcerations (lesions of superinfection) of the small intestine (specimen from Professor Curtis, Lille).

3. Tuberculosis of the large intestine in the adult. Tuberculous ulcers in plaques (specimen from Professor Curtis, Lille).

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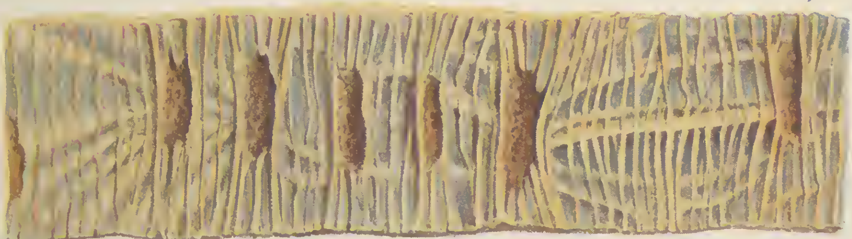

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Among 67,489 children treated in the hospitals of England and Scotland (*Edinburgh and Glasgow* not included), and among 68,488 children treated in hospitals on the European continent, the percentage of cases of abdominal tuberculosis is respectively 1.6 and 1.13; whereas, in American hosptials, among 37,129 children the percentage is but 0.28. On the other hand, in the cities of *Edinburgh* and *Glasgow* the percentage reaches the enormous figure of 3.9. It seems. therefore that in Great Britain, in a general way, abdominal tuberculosis is much more common than meningeal tuberculosis, while the contrary holds for the principal cities of the continent and also for the United States. At *Paris*, according to G. Variot, tuberculous meningitis is incomparably more frequent.

E. TUBERCULOSIS OF THE GLANDS OF THE NECK

The frequency with which tuberculous localizations occur in the glands of the neck in children is well known, yet they are never observed in the course of the first year and are only exceptionally encountered before the age of three years. Cervical gland tuberculosis is above all a disease of childhood, the period when the child puts its hands to every thing (tuberculose des *touche à tout*), appearing in subjects whose lymphatic system is already more resistant than that of young infants. It is the evidence and the result of a neighboring local infection, which may be of the ocular, nasal, buccal or pharyngeal mucosa, or, probably more commonly, of the tonsils. At times the glands have simply retained in their stroma meshes a few bacilli which have been phagocytized by the leucocytes and absorbed through the sub-mucous lymphatic network. It is a primary infection whose intensity and gravity are in proportion to the number and virulence of the bacterial elements. Again the bacilli gain entrance through a small wound or by virtue of a local infection by pyogenic microörganisms (rhinitis, otitis, pharyngitis, tonsillitis, gingivitis, adenoids, etc.).

Tuberculous infection of the cervical glands is generally followed by their more or less rapid engorgement. The glands are hard, movable, and only slightly painful. Among them there is always one much larger than the others of the same group. The lesion within it may remain shut off and inactive for months, later to retrogress and gradually disappear; or again it may progress toward caseation. If the subject has been absolutely free from tuberculosis

up to that time, it may become the point of departure for a generalized infection. If he has been partially immunized by another earlier and more benign involvement, a local cold abscess forms which heals readily by sclerosis on being evacuated either spontaneously or by operation.

Such are the principal anatomical characteristics presented by tuberculous infection in early childhood. It is only later, at the beginning or in the course of later childhood, rare instances excepted, that one observes the cutaneous, bone or joint localizations to be discussed in other chapters.

CHAPTER XII

PATHOLOGICO-ANATOMIC CHARACTERISTICS OF TUBERCULOUS LUNG INFECTION IN ADULTS AND IN THE AGED

It is a well known fact and one to which we shall often return in succeeding chapters, that in cities all over the world and even in small rural localities, particularly in Europe, tuberculous infection is so widely spread in the human race that very few individuals escape it before arriving at adult age.

Tuberculin reactions show us (*Chapter XL*) that in the centers of old civilization almost 95 per cent of individuals who have attained or passed the age of 20 years are the bearers of some focus of tubercle bacillus infection, which may or may not be extensive and which may be in process of evolution or may be "occult." Throughout their lives, which may, indeed, be of normal duration, these individuals for the most part will be ignorant of the fact that they are harboring bacilli or have done so.

The infectious elements which have gained entrance into their bodies, almost always in the early years of childhood, manifest their harmfulness only if they have penetrated *in sufficient number*, or on *several occasions within a short time*. And the effects produced depend upon: (1) their *degree of virulence* (according to the source—human or bovine, and the nature—pulmonary, intestinal, bony, etc., of the lesion whence they were derived); (2) *the places of localization*, which, 9 times out of 10 in the adolescent and the adult, are in the parenchyma of the lung; (3) *the susceptibility of the individual*, which is greater or less depending upon freedom from a previously acquired tuberculous infection, or upon a benign glandular infection, more or less in the past and latent, such as may render the body relatively *tolerant* to new infections.

Among the many individuals who harbor bacilli we shall see that the proportion dying from tuberculosis is considerable, since the figure varies from 12 to 32 and up to 35 per 100 deaths depending upon the place of abode;—city or country. But individuals who do not succumb to their tuberculosis in early or adult life and who suffer

from no apparent illness, will retain foci nevertheless up to extreme old age, foci which are active, latent or concealed, and which almost always contain living virulent bacilli.

The statistics compiled some time ago by Naegeli[1] indicated that 97 to 98 per cent of adult subjects, autopsied in hospitals, show tuberculous lesions. In the aged alone 60 per cent show them (Nat. Guillot). Brouardel at the Morgue in Paris, and Schang and other observers arrived at approximately the same conclusions.

If the pathologico-anatomic forms of tuberculosis presented in the adult and the aged differ from those observed in infancy, or from those seen in negroes transported to Europe, the reasons are, first, that in adults and the aged we have almost always to do with *reinfections* and not with *primary infections*, and second, that the lymphatic system, altering its structure after the third year, has no longer the same ability to retain and "cultivate" the tubercle bacillus within it.

A. ACUTE MILIARY TUBERCULOSIS OF THE LUNGS

In the fairly rare instances where the subject, no matter how old, is entirely free from a previous tuberculous infection, his susceptibility, although less than that of the nursling, is such that a rapid invasion, with the clinical picture of an *acute miliary phthisis* will occur in the presence of a sufficiently ample and virulent infection.

This particularly grave form is characterized anatomically by the presence of numerous gray miliary tubercles in the lung, the pleura and most of the viscera. The lungs are congested and hard; they have lost their elasticity. Against a greyish pink or red background a large number of small semi-translucent miliary tubercles the size of a pin head, are to be seen and, in places, there are foci of broncho-pneumonia (*figs. 10 and 11*).

The pleura likewise is covered with granulations and very thin false membranes, fibrinous in character. At times a little serous or hemorrhagic effusion is to be found.

The miliary tubercles are rarely restricted to the organs of the thorax. They extend usually to the various serous membranes (meninges, pericardium, peritoneum, joints), to the spleen, liver and kidneys, and to the endothelium of the vessels.

[1] Virchow's Arch., 1901, **160**, 426.

B. ACUTE PNEUMONIC TUBERCULOSIS

Frequently enough a tuberculous infiltration resulting from massive infection in a tuberculosis-free individual manifests itself from the beginning and exclusively as a pneumonia. This condition is *acute pneumonic phthisis*, and usually involves but a single lung, a few lobules of a single lobe, or one entire lobe. It is more frequently on the right side than on the left and at the base than at the apex. It may be suspended as it were between two halves of healthy lung tissue (*see Plate IX*).

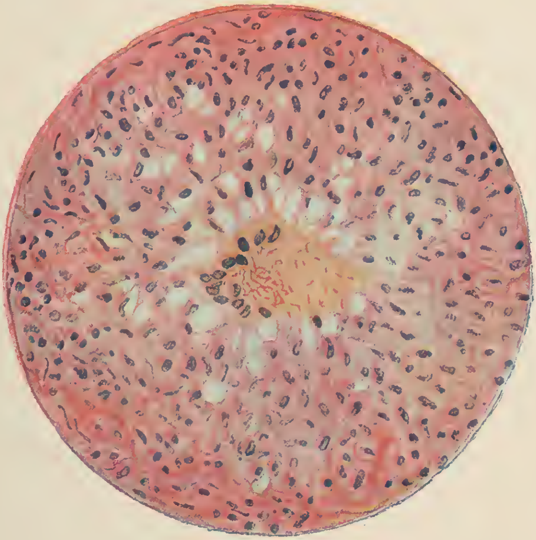


Fig. 10. Miliary Tuberculosis of the Lung

Giant cell. 1mm. $\frac{1}{12}$, oc. comp. 6, Zeiss.

In the affected area the alveoli are obliterated by a caseous mass of the color and appearance of Roquefort cheese. About the caseous foci the tissue is infiltrated with gray translucent substance (*infiltration grise* of *Laennec*), often gelatinous (*infiltration gélatiniforme* of *Laennec*), in which there stand out small opaque masses, yellowish white, which represent a step in the caseous degeneration.

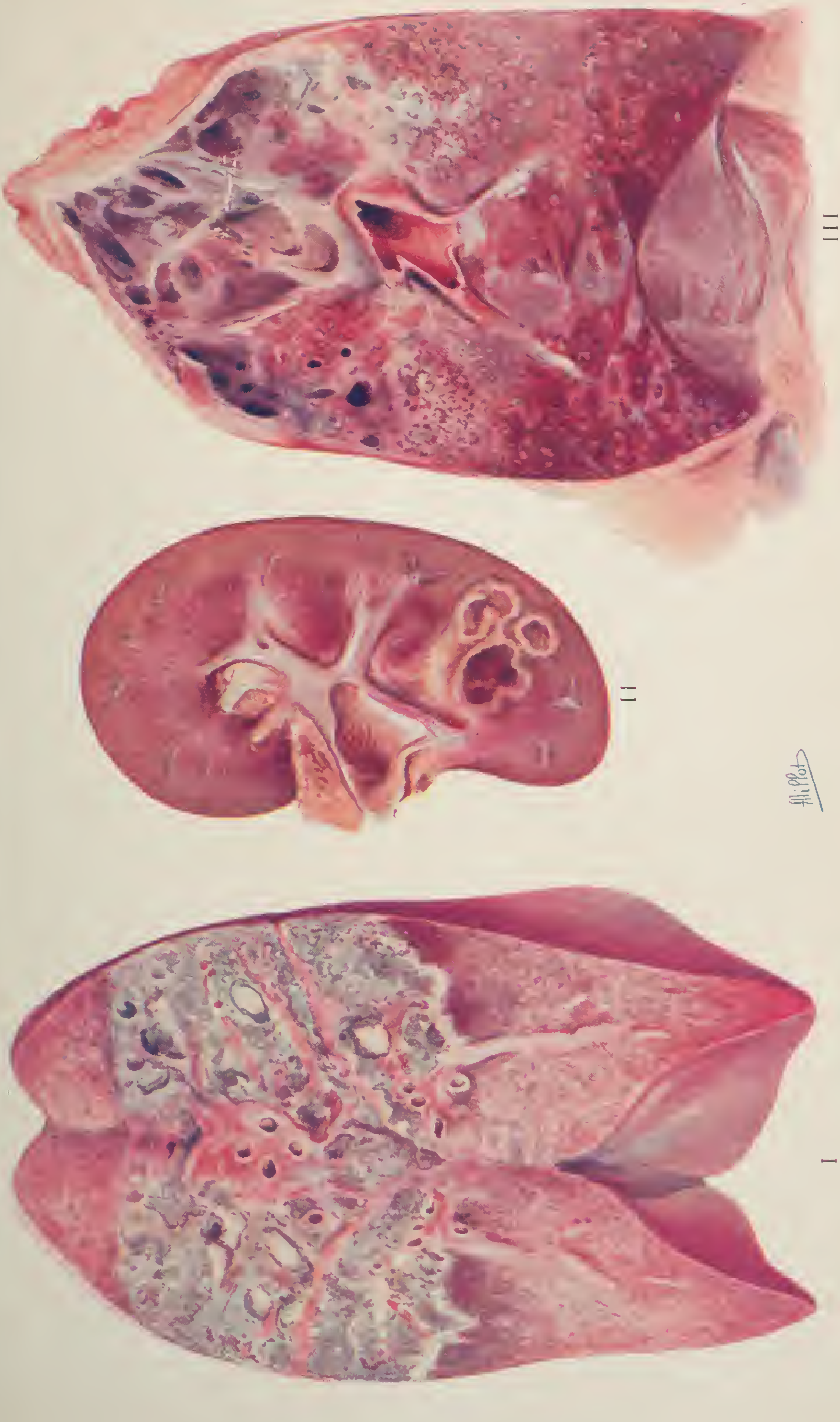
About the isolated areas of pneumonia, the lung is more or less congested, infiltrated and emphysematous. These changes find their way to the overlying pleura.

PLATE IX

1. Pulmonary tuberculosis (suspendue). So called on account of its being suspended as it were in relatively normal lung tissue. Tuberculous nodules and multiple small cavities.—Anthracotic sclerosis. Apex intact. (Specimen from Professor M. Letulle.)

2. Renal tuberculosis. Cavities in the lower pole of the kidney. (Specimen from Professor Curtis, at Lille.)

3. Pleural symphysis. Multiple partitioned cavities of the apex. Disseminated tuberculous nodules (from an autochrome photograph of a specimen from Professor Letulle).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It is generally agreed today, with Grancher, Hutinel, Aviragnet, Mosny, and others, that acute pneumonic phthisis is caused by a mixed infection of the tubercle bacillus and the pneumococcus or a streptococcus. The secondary pyogenic infection, grafting itself upon a focus of pulmonary tuberculosis which is recent and in process of evolution, is supposed to induce a massive infiltration of the hepatized tissue by a veritable culture of bacilli.

Acute pulmonary phthisis, with its fatal prognosis in persons free from a previous tuberculous infection, may attack individuals rendered resistant to tuberculous reinfection by an old and benign

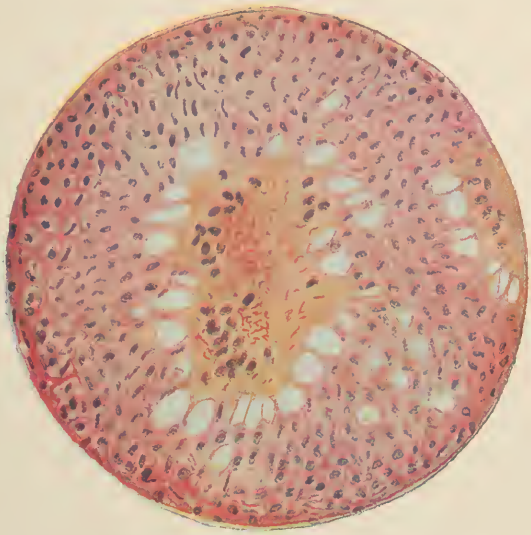

FIG. 11. MILIARY TUBERCULOSIS OF THE LUNG

Two fused giant cells in a tuberculous nodule. 1mm. $\frac{1}{12}$, oc. comp. 6, Zeiss

infection. It then exhibits a remarkable tendency to heal, through the softening and elimination of the focus in the form of a *vomica.* Cavities result which are capable of cicatrizing themselves through a sclerosis of their walls.

C. CHRONIC PULMONARY PHTHISIS

But the form of tuberculosis which is really characteristic of those subjects who possess a partial immunity to fairly often repeated and more or less massive reinfections,—or rather who possess that

particular state of intolerance to the tubercle bacillus which characterizes antituberculosis immunity (*see Chapter XXXIX* and following) —is *chronic pulmonary phthisis* or common *phthisis.*

The latter results from the development in the lung of one or more secondary foci or tubercles which tend to expel their contents, as every already-tuberculous body tends to expel a new dose of bacilli inoculated subcutaneously. In experimentation this is called the *phenomenon of Koch.*

The condition may disclose itself stealthily and slowly as the remote echo of a benign infection contracted in childhood and remaining latent for years (most often in some gland of the mediastinum). Ordinarily it is then not serious. It begins and lodges itself almost always at the apex, on the right by preference, and manifests itself clinically by signs indicating the presence of small foci of caseated peribronchial tubercles. This form ends usually in cicatrization by sclerosis; it is the *abortive tuberculosis* of *the apex* (*tuberculose abortive du sommet*) (Bard).

Much more often the case becomes one of *chronic pulmonary phthisis with ulcerative tendency,* which slowly eats away and excavates the lung in proportion as the organism tends to expel the tubercles which break out as the result of repeated auto-reinfections or reinfections from without (*phenomenon of Koch*).

This process of elimination begins in an early focus of nodules, peribronchial, perialveolar or sub-pleural, which has undergone caseous degeneration and then become softened. The process meanwhile continues to excavate peripherally until the contents find an outlet into a bronchus. The alveoli to which this bronchus leads become dilated before being included themselves in the process of cellular destruction. As a result there are formed small cavities (*acinous cavities*) which enlarge to become *lobular cavities,* then *multilobular,* then *lobar,* when they extend to one or several lobes.

The size of these cavities may vary greatly, from that of a pea to that of an orange, or even larger. They extend as a rule from the top of the lung toward the base, and are often divided into convoluted compartments by partitions or incomplete loops of torn and sclerotic tissue. They are filled with sero-caseous pus mixed more or less with thick, bronchial, stringy mucus. Their festooned walls almost always organize to form a fibrotic shell, fixed to the neighboring pulmonary tissue, which itself is indurated, or to the pleura whose

two layers become thickened and adherent to each other at the corresponding level. Between this fibrotic shell and the portions of the lung which remain normal there exists constantly a zone of interstitial pneumonia (*Plate X*).

In the walls of the cavity there are found at times small pedunculate pear-shaped aneurysms of the branches of the pulmonary artery or of the bronchial arterioles; they are the so-called *aneurysms of* Rasmussen, having been described by him in 1868. Their formation, according to Eppinger and Ménétrier, results from the fact that the arterioles included in the walls of the cavities themselves undergo an infiltration with tubercle bacilli and become destroyed layer by layer from without inward, so that the vessel finally consists of nothing but its intima. Blood pressure then gradually causes the formation of an aneurysmal sac whose resistance ultimately yields, and whose rupture gives place to the large hemoptysis of the cavity stage.

Tubercle bacilli are always present in immense numbers in the cavity contents; particularly abundant in the cheesy matter which covers the walls. When the latter become sclerotic and the secretions dry up, the bacilli decrease in number and finally disappear. But cavities which are at all extensive rarely heal by sclerosis. Fibrous transformation is almost never complete. On a fibrotic and more or less retracted flooring, there remain a few caseous or calcifying nodules in which the bacillary elements persist indefinitely.

Dissemination of tuberculous lesions in different parts of the lung is brought about,—either by direct extension through the intermediary of the lymphatic vessels which are spread thickly around the alveolar acini, the bronchioles, and the bronchi, and in which multiple small foci of tuberculous lymphangitis develop,—or by auto-reinfection subsequent to absorption, through the intestinal or bucco-pharyngeal mucosa, of bacilli evacuated from the cavities with the sputum. Thus is explained the fact that at the autopsy of a phthisical case one always finds tuberculous lesions in all stages, from large cavities down to gray, still translucent granulations.

Most frequently the lesions exist only in one lung or in both lungs, extending locally until there no longer remains enough healthy tissue to assure the indispensable minimum of hematosis, but they do not invade other viscera. It is remarkable how, in the adult, the tracheo-bronchial gland group remains apparently free from infection, although the pulp of the glands always contains many bacilli.

PLATE X

1. Chronic pulmonary tuberculosis with large and small cavities and anthracosis (specimen from Professor Curtis at Lille).

2. Chronic pulmonary tuberculosis with large partitioned cavity of apex (specimen from Professor Letulle).


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And yet at times the process does disseminate itself—always by the lymphatic channels—to other neighboring or distant organs. Thus tubercles are seen in the larynx, producing superficial or deep ulcerations, located most often in the interarytenoid region. The ulcerations may invade the vocal cords and the epiglottis, and there even develop vegetations made up of embryonic cells and of miliary tubercles covered with stratified epithelium.

These lesions of the larynx are frequent in individuals affected with chronic phthisis, and they render the prognosis more grave by hastening the evolution of the disease, at the same time causing the case to be more dangerous from the point of view of dissemination of the bacilli, through provoking almost incessant coughing.

D. PULMONARY TUBERCULOSIS IN THE AGED

In the aged, contrary to what was believed until recently, chronic pulmonary tuberculosis is very frequent. It often takes that particular form which clinicians call essential asthma or emphysema (Hirtz). One should always suspect this illness, all the more since it usually goes unrecognized by reason of its insidious and mild symptoms. It tends to be without fever and to progress extremely slowly.

From October, 1909, to December, 1910, at the Departmental Home of the Department of the Seine, R. Oppenheim and Ch. Le Coz[2] autopsied 260 subjects of more than 60 years. In 193 of these cases they found lesions of pulmonary tuberculosis; healed in 110 instances and progressive in 83.

According to the pathological evidence as to the causes of the deaths, it would appear that 46, or 17.7 per cent of these 260 elderly people died of tuberculosis, whereas 193 or 74 per cent of them were, at some period of their lives, infected with tubercle bacilli.

During a period of one year, the majority of the aged examined by the same observers were tested intradermically with tuberculin. Among 1162 individuals the reaction was positive in 77 per cent, a figure very close to the 74 per cent given as the proportion of the aged who at autopsy showed manifest lesions of pulmonary tuberculosis.

In compiling the statistics for the city of Paris for 1910, Landouzy[3] found that manifest senile tuberculosis was far from exceptional.

[2] Progrès méd., 1911, **27**, 5.

[3] Bull. Acad. méd., 1913, **69**, 463.

Of 16,229 persons dying of tuberculosis in the Department of the Seine, 1317, or 8.11 per cent, were more than 60 years old; and of 23,251 aged subjects who died during the same year in the same Department, 1317, or 5.66 per cent, succumbed to tuberculosis.

Baudot[4] extended the inquiry over a period of 10 years,—following the Annual Statistical Report of Paris,—and showed that in the course of this time, among 149,566 deaths from all causes in subjects older than 60 years, 8276 were due to various forms of tuberculosis, of which 7507 were pulmonary phthisis. During the same period of time, for the same category of individuals, the Department of the Seine showed 12,304 deaths from cases diagnosed as tuberculosis and 10,020 deaths from chronic bronchitis, in a total of 234,538 deaths. Without doubt a large part of the deaths attributed to bronchitis should in reality be charged to tuberculosis.

The virulence of the bacilli derived from pulmonary lesions in the aged is certainly as great as that of bacilli isolated from other phthisical patients, as proved by the experiments of J. Courmont and Revol.[5] This fact is likewise established by innumerable observations of family contagion reported by many authors and pointed out repeatedly by such eminent clinicians as Jaccoud, Potain, Landouzy and Déjerine. In studying the causes of infantile mortality, Landouzy cited some striking examples of such contagion within the family. In the case of the tuberculous infants in the nursery of the Hospital Tenon, it was generally easy for him to find evidence of tuberculosis in the lineal ancestry, and the guilty one was often the grandmother who cared for the baby while the parents were away at work in factory or shop.

But a case which he presented before the Academy of Medicine is still much more convincing.

"In a luxurious home in the Champs-Elysées and under ideal conditions there resided a foreign family, composed, in addition to a considerable number of servants, of the father and the mother, who had married at the ages of 28 and 20 years respectively, and who were both in the best of health and had excellent past histories. The mother had given birth successively, after normal pregnancies at intervals of about two years, to three fine boys whom she brought up exclusively at the breast.

[4] Thèse, Paris, 1913.

[5] Bull. de la Soc. méd. de Lyon, 1904, **3**, 137.

"Aside from the regular feeding hours, the babies were left to the care of a governess, a thin delicate woman, greatly devoted to the family, four generations of whom she had seen grow up.

"The three boys, one after another, almost at the same age, died of tuberculous meningitis. I was present at the third of these deaths, which the physicians, Jules Simon and Archambault, said was simply a repetition of what they had seen in the cases of the older brothers.

"I set about to discover the cause of the three cases of meningitis which seemed a challenge to the hygienic conditions, comfort and health which reigned in the home.

"The investigation led to my finding in the governess, who was more than 60 years old and a sufferer from catarrh, chronic bronchitis, emphysema and asthma formerly, a type of those torpid senile tuberculoses which are suspected all the less because such neuro-arthritic patients, by virtue of their being always active, never giving up, and looking far from ill, preserve the appearance of resistance at least, if not of health.

"I arranged that the governess should be sent back to America, but not without difficulty since she had been attached to the family for 50 years. After her departure nothing in the home was changed. Two children were born and were nursed exclusively by the mother. One is now approaching 30 years, the other is a superb girl of 25 years.

"If, in such favorable surroundings," adds Landouzy, "senile tuberculous infections are capable of contaminating the whole of a woman's domain, how much more terrible must they be in overcrowded rooms and in the home of the laborer where babies, children, father, mother and grandparents, live one on top of another, as the popular saying goes!"

Such facts, carefully noted, have as much value as laboratory experiments. They should be kept in mind and duly considered when a scientific basis for tuberculosis prophylaxis is to be laid down.

CHAPTER XIII

TUBERCULOSIS OF THE SEROUS MEMBRANES

A. TUBERCULOUS PLEURISIES

Tuberculous infection of the pleura is always a secondary manifestation. It may however occur in a subject in whom lymphatic infection is quite recent, when the pathological effects are rather severe, but usually, they are benign. The form of pleurisy observed in such a case is *acute pleurisy with or without sero-fibrinous effusion.*

When the infection occurs in a subject already tuberculous and more or less intolerant toward the tubercle bacillus, the invasion of the pleural sac provokes a natural effort to expel the organisms and a purulent effusion is formed like that of a cold abscess. This is suppurative pleurisy.

It often happens that a lesion of the apex of the lung precedes the appearance of a pleurisy, or the latter follows a tuberculous infection of the glands of the hilum or of the mediastinum. It may also result from a tuberculous infection of the tonsils. According to Grober,[1] if one injects India ink into a tonsil, the colored particles are transported by the lymph current to the corresponding pleura and they may be recovered in the glands of the hilum and in the supra-clavicular glands which drain the pleural lymph.

Following traumatisms of the thorax, even though slight, sero-fibrinous pleurisies are frequently seen to develop in tuberculous patients. According to Netter, 68 per cent of pleurisies are caused in this manner. Pleurisy may also occur subsequent to tuberculous infection of the peritoneum, or in individuals with lesions of the vertebrae or ribs.

1. Acute sero-fibrinous pleurisy

Sero-fibrinous pleurisy is noted but rarely in young children. It does not become common until after the fifth or sixth year and is

[1] Deutsch. Arch. f. klin. Med., 1902, **74**, 43.

particularly frequent about the age of 20 years. It attacks chiefly individuals who have no evident past history of tuberculosis. From the pathologico-anatomic standpoint, it is characterized by congestion of the blood vessels of the pleura, by an eruption on its surface of a great number of fine granulations or vegetations which give it the appearance of shagreen or of the tongue of a cat, and by an exudate composed of serous fluid containing very delicate reddish false membranes which at times thicken by budding and which spread like a leather membrane over the whole affected area. In this region the pavement epithelium desquamates and the subjacent layer of connective tissue becomes infiltrated with embryonal cells and a great number of lymphocytes. Tubercles are here found either isolated or in more or less dense masses.

The false membranes have the consistency of coagulated fibrin. They form flakes and strands adherent to the serosa or floating freely in the fluid. In their network are held numerous leucocytes, a few disintegrated pigmented cells, some red blood cells and a large number of lymphocytes.

The exudate, ordinarily of a brownish yellow color, transparent or slightly turbid, is composed of blood plasma and holds in suspension a few filaments of fibrin, some red cells, leucocytes, lymphocytes and a small number of bacilli which can be demonstrated only by experimental inoculation. Its amount varies greatly, from about 200 cc. to 4 litres and at times even more. Its albumin content varies from 1 to 1.5 per cent. When collected in a glass vessel a jelly-like mass is formed after a few hours.

The lung tissue underlying the pleural lesions is found congested, infiltrated with fibrin and leucocytes, at times consolidated, or even carnified in rather old pleurisies.

Sero-fibrinous pleurisy rarely affects the whole of one pleura; most often it is localized either at the base in the diaphragmatic region, in the middle portion, or at the apex. It may also be interlobar solely. Effusions may then form in the spaces walled off by the false membranes thick enough to maintain them.

In the dry forms no exudate is produced, but the lesions are the same. They often accompany attacks of so-called cortical splenopneumonia (*spleno-pneumonia corticale*) which are fairly frequent in pulmonary tuberculosis.

When healing takes place, which is almost always the rule,—unless the pleurisy marks the beginning of a generalized tuberculosis,—the fluid gradually reabsorbs, if not too abundant or withdrawn by thoracentesis. But the pleura remains definitely altered; adhesions form between the two layers through the fusing together and fibrous transformation of their tuberculous vegetations. The adhesions may thicken to the extent of forming "pleural symphyses." Nevertheless the patient suffers no great inconvenience, and clinicians have long since observed that sero-fibrinous pleurisy does not as a rule seriously compromise the ultimate prognosis. It represents a rather benign form of tuberculosis, tending to heal spontaneously by fibrosis and conferring upon the affected individual a manifest resistance to reinfection. An old pleurisy case never contracts an acute miliary tuberculosis afterward. He may become phthisical and this occurs quite often if he exposes himself to frequent and massive infections; but the disease then assumes one of the torpid forms compatible with work and the appearance of relatively good health during many years.

The extreme frequency of *sub-pleural* tuberculous nodules, especially the anthracotic, more rarely the calcareous type has been repeatedly noted. M. Letulle demonstrated that these isolated "colonies" of bacilli, constituting veritable emboli in the subpleural vascular connective tissue, are formed in reality in the "rudiments" (*esquisses*) of lymphatic glands, the nodular points of reticular tissue which are scattered through the deep layer of the visceral pleura. They represent "a sub-pleural tuberculous adenitis."

These anthracotic sub-pleural nodules constitute the typical tubercle in process of healing.

2. Suppurative pleurisy

It is known today that although sero-fibrinous pleurisy is, in the majority of cases, the manifestation of a tuberculous infection of the pleura, suppurative pleurisy may result from infection by other bacteria, among which the streptococcus is found most frequently (50 to 60 per cent), at least in adults, while in children it is said to be the pneumococcus (74 per cent).

Tuberculous suppurative pleurisy is however fairly common, although observed only in definitely tuberculous cases, either following a sero-fibrinous pleurisy or taking its form from the outset in an

already phthisical individual or one who has serious and long-standing lesions of other organs. The condition is always fatal after an interval more or less brief.

In this form of tuberculosis the pleura, especially the parietal layer, is covered with caseated and ulcerated tubercles. It is greatly thickened, up to one centimeter or even more at times. The lesions extend in general in large plaques over the whole of one side. They originate often from a tuberculous mediastinal or intercostal gland.

The pus which characterizes these cases resembles that of a cold abscess. It is granular, greenish or yellow, and contains no fibrin. Bacilli are rare; but their presence may always be demonstrated by experimental inoculation into the guinea pig. A. Fraenkel was able to state that whenever culture or microscopic examination of a purulent pleural fluid reveal no pyogenic microörganisms, the case is one of tuberculous pleurisy. It is not even necessary then to await the result of the inoculation in order to make the diagnosis.

When caseous tubercles of the lung break through the pleura in their neighborhood and there is a coexistent suppurative pleurisy, a communication is established between the lesions. and a hydro-pneumothorax develops.

B. TUBERCULOUS PERITONITIS

Infection of the peritoneum may be brought about by bacilli discharged into the circulation through the rupture of a caseated primary tuberculous lymph node. Such an infection may manifest itself as an *acute miliary peritonitis*, or it may develop slowly in an individual who already has multiple tuberculous lesions, and give the clinical picture of *chronic ulcerative peritonitis* or of *fibrous peritonitis*.

Acute peritonitis occurs rather frequently in women, and at times also in men, following tuberculous infection by the genital tract or a rapid extension to the peritoneal lymphatics of a recent infection whose primary seat is located in the genital organs.

In most cases however it results from the discharge into the serous cavity of a mass of tubercle bacilli derived from a caseated tubercle which may have been situated, either in a mesenteric gland, or an iliac or diaphragmatic gland (in relation or not with an acute sero-fibrinous pleurisy or with an acute coexisting tuberculosis of the mediastinum), or even in some lesion of an organ more distant.

For a long time it was believed that peritonitis usually followed a tuberculous enteritis, and experiments of Baumgarten and Orth who had reproduced the disease in animals by having them ingest, simultaneously, hard objects capable of injuring the mucosa and bacillus-containing material, seemed to confirm this point of view. But the clinical observations of Spillmann, and then the experimental work of Dobroklowsky,[2] of von Behring, and that which I have published with my pupils C. Guérin, M. Breton, and others, have shown that intestinal absorption of tubercle bacilli takes place without leaving the slightest trace of the passage of the bacilli through the mucous wall, and that lesions of the latter, when they do exist, are secondary, subsequent to the peritonitis, representing simply the extension of the tuberculous process to the closed follicles and to the peri-intestinal lymphatic glands.

In tuberculous peritonitis as in pleurisy,—which it very frequently accompanies since the two serous cavities are contiguous and communicate through anastomosis of their lymphatic vessels,—disseminated miliary tubercles are found in large number and often in large fibrin-covered sheets on the surface of the serosa. The latter assumes the appearance of shagreen and the peritoneal cavity is filled with a sero-albuminous fibrin-containing fluid of a greenish yellow color. This fluid, which is slightly cloudy, contains a few red blood cells, a fair number of leucocytes, many lymphocytes and a very few bacilli which are generally to be found only after centrifugation or by animal inoculation. Its quantity may attain to as much as 8 litres, but as a rule there is less. In acute miliary tuberculosis of the peritoneum, other organs (lungs, liver, spleen, kidneys, etc.) are almost always affected.

Ulcerative peritonitis presents the same lesions, but with greater local development. The plaques of tubercles are caseated, suppurating and fitted into septa of coagulated fibrin which imprison many leucocytes and form pockets. The fluid enclosed in the latter is thick, grumous, and brownish in color. At times the lesions condense and encyst themselves by glueing together the organs involved, for example those of the true pelvis (pelvic-peritonitis). The fluid may be resorbed and the membranes undergo fibrous transformation; or the ulcerations may extend, destroy the intestinal wall and cause fatal perforations.

[2] Arch. de méd. expér., 1890, 2, 253.

In *fibrous peritonitis* the tubercles make their appearance more deeply in the sub-endothelial connective tissue. They have little tendency to develop and caseate. The serosa which covers them loses its lustre. More or less fluid accumulates in the peritoneal cavity and a fibrinous exudate tending to become organized spreads itself over the surface. Bacilli are here extremely rare.

Little by little cicatrices are formed which retract the lesions into round forms with depressed centres, or into fibrous plaques. Apparently there is healing. But ordinarily the mesentery and omentum become cordlike. The strands may strangulate the intestinal loops and the shrunken mass of gut may undergo atrophy by taking part in the process of sclerosis. The consequences are serious for both the nutrition and life of the patient.

C. TUBERCULOUS PERICARDITIS

As regards pericarditis, one may repeat what has already been said as to pleurisy and peritonitis. There is an acute pericarditis resulting from a recent infection limited to the mediastinal glands adjacent to the pericardium,—and it may be, up to the death of the individual, the sole manifestation of tuberculous infection, or again it may be accompanied by other localizations in neighboring or distant organs. There is also a pericarditis which appears in long-standing tuberculosis cases, whether glandular, bone or pulmonary, who harbor localized or disseminated caseated lesions in various organs.

Thus acute tuberculosis of the pleura and of the peritoneum frequently extends to the pericardium, the bacilli being transported by the lymphatic channels by means of which these three serous cavities communicate freely with one another.

The anatomical lesions are always of the same sort; they may be dry and terminate in symphysis, or be accompanied by effusion which is often hemorrhagic, with more or less thick fibrinous false membranes, lamellar in arrangement and forming cellular spaces. Miliary granulations and caseous tubercles develop chiefly in the epicardial layer. They rarely go on to fibrosis. Almost always they are followed in a short time by death, with granular fatty degeneration of the myocardium.

CHAPTER XIV

TUBERCULOUS MENINGITIS AND TUBERCULOSIS OF THE NERVOUS CENTRES

A. TUBERCULOUS MENINGITIS

Tubercle bacillus infection of the lymphatics or blood vessels of the pia mater, which is characteristic of tuberculous meningitis, is never primary. It may exceptionally be produced by direct extension of the virus from a lesion located near the brain, for example in the ear, the nose, the eye, the maxillary sinuses, or from caries of a vertebra (*Pott's disease*). Most frequently, however, it is the result of a pouring into the general circulation of a quantity of bacilli through the rupture of caseated tubercles in the tracheo-bronchial or mediastinal lymph nodes. This is the only pathogenesis, with rare exceptions, in the child, although, in the adult, the infection may originate in any sort of suppurative tuberculous lesion which at a given moment evacuates a sufficiently large number of bacilli into the blood stream.

Although never the result of a primary infection (except in *acute generalized miliary tuberculosis* in which it is but an incident), *tuberculous meningitis,*—at least those forms which cause death in a short time, and these are the most numerous,—*develops only in subjects recently and intensely infected* and who have not yet acquired, even partially, the least immunity against tuberculosis.

Tuberculous meningitis assumes at least three clinical and anatomical types which are fairly clearly differentiated: that of early life or the *common type of tuberculous meningitis, miliary tuberculosis of the meninges* and *meningitis in localized areas* (*en plaques*). Other and rarer forms exist, such as the atypical non-follicular meningitis, described by Landouzy and Gougerot, meningitis localized in the bulb, for example, or in the spinal cord (spinal meningitis), and the forms which Tinel and Gastinel[1] call meningeal states (*états meningés*) and which are attenuated forms of meningitis capable of cure.

When a child dying of common tuberculous meningitis is autopsied and the dura mater incised, the brain is found bathed in sero-

[1] Rev. de méd., 1912, **32**, 241.

sanguineous fluid. The venous sinuses are dilated and the pia mater is thickened and gelatinous.

On disengaging the brain from the base of the skull the characteristic lesions grouped *around the optic chiasm* and *at the beginning of the fissure of Sylvius* are seen. They are covered by a sero-purulent exudate and are formed of granulations or of tubercles in different stages, usually collected together in clusters in the thick portion of the pia mater. The lesions may spread along the vascular sheaths which extend into the fissures of the whole cerebral mass, but they are localized ordinarily at the base about the *circle of Willis* as well as along the *fissure of Sylvius*, and involve the lymphatic sheaths of arteries or arterioles in whose walls they set up inflammatory cellular reactions. These reactions result in lesions of perforating, proliferating and obliterating arteritis. Round about these lesions the blood infiltrates to form small hemorrhagic areas in the pia mater and at times in the sub-arachnoid spaces.

The nervous tissue of the brain is found adherent to the meninges which strip off with difficulty. The penetrating vessels show perivascular lesions, and their lymphatic sheaths are infiltrated with leucocytes, lymphocytes and mononuclears particularly.

The cellular changes occur principally in the large cortical pyramidal cells which undergo chromatolysis. Their nuclei, moreover, become eccentric.

In the ventricles there are often observed congestive and granular lesions of the choroid plexus and of the ependyma. The ventricular fluid is more abundant than normally and tends to produce an edematous infiltration of the brain (*ventricular hydrocephalus*).

The spinal cord and its covering membranes are frequently involved in the process of tuberculous meningitis. Vascular lesions or tuberculous granulations are then found widely disseminated or grouped over the surface of the enlargements and in the posterior longitudinal fissure, especially in the lumbar region.

In miliary tuberculosis of the meninges (*primary infection*), miliary granulations are extremely numerous, remain grey and translucent and do not develop to the point of forming tubercles. The perivascular infiltration and the alterations resulting therefrom have scarcely time to more than outline themselves.

In meningitis in plaques, on the contrary, the tubercles are collected in a mass, at times as much as one centimeter thick. They are

caseated or tend to calcification or sclerosis, and are usually flattened out in the region of the fissure of Sylvius.

Among the atypical varieties, the non-follicular meningitis of Landouzy and Gougerot[2] (*bacillémie à forme méningée de Debove*) is revealed only by the presence of tubercle bacilli in the cerebro-spinal fluid, whether after death or by lumbar puncture during life. The only apparent lesion is a congestion of the meninges with an accumulation of turbid exudate, full of lymphocytes and mononuclears principally along the vessels at the bottom of the parietal lobe fissures.

In tuberculous meningitis the cerebro-spinal fluid, withdrawn by lumbar puncture, is ordinarily clear, of a faintly greenish tinge, with only a little fibrin, but enough to form a coagulum after standing a few hours.

It contains about 7.43 gms. of sodium chloride per liter according to Nobécourt and Roger Voisin; only 5 to 6 gms. according to Mestrezat and Gaujoux.[3]

The predominating cellular elements in this fluid, as shown by Widal, Sicard and Ravaut, are the lymphocytes. According to Netter and Gendron,[4] the lymphocytosis in tuberculous meningitis of children varies from 100 to 160 per cubic millimeter. But there are found some more or less altered polynuclears, mononuclears and eosinophiles. A. Lutier[5] states that lymphocytes predominate in 88 per cent of the cases and polynuclears in 14 per cent.

Tubercle bacilli are seldom present in large number. In searching for them, the fluid withdrawn by lumbar puncture should be immediately centrifugated, before coagulation, and preparations of the sediment stained with Ziehl. If the puncture is made with the proper degree of asepsis the sediment may be planted upon glycerin serum agar or on the egg media of Dorset and of Lubenau to obtain a pure culture for a study of the virulence. The human or bovine origin of the infection may thus be determined. Or again, after centrifugating one may inoculate the sediment directly into the peritoneum or under the skin of a guinea pig, or else, and this is preferable, one may follow V. Grysez at the Pasteur Institute at Lille and inject the sediment, after centrifugating, into the spinal canal of a tuberculous

[2] Thèse, Paris, 1909.
[3] Compt. rend. Soc. de biol., 1909, **66**, 424; 533; 637.
[4] Bull. Soc. de pédiat., 1911, **13**, 226.
[5] Thèse, Paris, 1903.

guinea pig infected 4 to 6 weeks previously. If the fluid is actually from a case of tuberculous meningitis the animal soon shows a hypothermia and dies in 4 to 6 hours. The diagnosis of the nature of the disease is thus made very quickly.

Vincent[6] has proposed another method called the *precipito-diagnostic,* based on the principle that on adding a little spinal fluid *in vitro,* to a small quantity of crude tuberculin and leaving them in the incubator for two or three hours, there is produced a sediment which is not obtained with normal fluid nor with that of epidemic cerebro-spinal meningitis. But I have demonstrated with L. Massol that this precipitin reaction is not specific.

Many attempts have been made to reproduce tuberculous meningitis, experimentally, but never successfully under satisfactory conditions. Thus Cornil and Bezançon, Péron,[7] then Sicard[8] injected pure cultures of tubercle bacilli into the carotid vessels of young dogs 4 to 6 weeks old. Where care was taken to previously ligate the two internal jugular veins two dogs among six are said to have shown typical symptoms and lesions (cerebral phenomena, vertigo, titubation, excitation, amblyopia), and to have succumbed 3 to 4 weeks after injection.

Louis Martin and A. Vaudremer[9] made attempts of the same nature using the guinea pig and rabbit. By inoculating directly under the dura mater they succeeded in producing, in 9 to 15 days in the guinea pig and in 5 weeks to 2 months in the rabbit, tubercles along the vessels and a gelatinous edema anterior to the cerebral peduncles, as in the child. But all of these experiments were upon non-tuberculous animals, so that the infection did not remain localized in the brain but became immediately generalized. The experiments should be repeated, but with animals which already have a glandular tuberculosis, instead of with normal animals.

Armand-Delille[10] attempted to differentiate upon the meninges the action of the different poisons produced by the tubercle bacillus. He showed that the ethero-bacilline, the chloroformo-bacilline of Auclair and the xylo-bacilline prepared by A. Borrel, provoke local cellular reactions of caseation or sclerosis, leading to hyperplastic

[6] Compt. rend. Soc. de biol., 1909, **67**, 765.

[7] Arch. gén. de méd., 1898, **182**, 412; 567.

[8] Presse méd., 1900, **1**, 67.

[9] Compt. rend. Soc. de biol., 1898, **50**, 273; 1067.

[10] Thèse, Paris, 1903.

new-growths which cause mechanical disturbances; but these poisons do not act upon the nerve cells. On the contrary, tuberculin and the bodies of the bacilli, when freed with xylol of their waxy fatty material, have an evident elective affinity for the elements of the nervous tissue. This fact had already been demonstrated by the experiments of Borrel[11] who showed the extreme toxicity of tuberculin for the tuberculous guinea pig on intra-cerebral injection.

It seems evident therefore that the functional troubles which characterize tuberculous meningitis are due in large part to toxic phenomena which result from the fixation of a greater or lesser quantity of tuberculin by the nervous cells adjacent to the miliary or nodular lesions.

Tuberculous meningitis is very frequent in children during the first year, indeed up to the 7th year. After the 10th year it becomes rare. Lesage and Abrami report that in all the cases which they have observed, three-quarters were in children of less than three years, and more than half in children from two months to two years. In the adult, the majority of cases is found between the ages of 20 and 25 years, but the incidence is incomparably lower than in childhood.

This form of tuberculous infection results almost always from contagion in the home. From this point of view W. Grunberg[12] made a comparative study of the mortality among the children in 568 families. Where the parents were healthy, the meningitis mortality of the children from one to three years was 1.7 per cent whereas in tuberculous families it was 10.8 per cent. And of 209 deaths from tuberculosis from birth to 15 years, he gives the following figures:

	BIRTH TO 1 YEAR	1 TO 3 YEARS	3 TO 7 YEARS	7 TO 15 YEARS
Meningitis	82	42	20	6
Tuberculosis of the lungs	6	19	12	7
Other forms of tuberculosis	3	4	3	5

After 15 years tuberculous meningitis becomes relatively rare, while the pulmonary forms appear much more frequently. Steinmeier[13] collected the statistics of the hospital of Eppendorf-

[11] Compt. rend. Soc. de biol., 1900, **52**, 358.

[12] Thèse, Paris, 1912.

[13] Virchow's Arch., 1914, **216**, 452. The same author has noted that in 7.57 per cent of subjects, tuberculous meningitis is related to uro-genital tuberculosis.

Hamburg from 1911 through 1913 and found that whereas children up to 15 years furnish 37.09 per cent of the cases, there are but 5.63 per cent of cases among adolescents and adults.

It is seen therefore that in the human race, the meningeal localizations of the tubercle bacillus, by their frequency and their gravity, dominate the whole of childhood pathology.

B. TUBERCULOSIS OF THE NERVOUS CENTRES

Tuberculous lesions developing in the brain and spinal cord generally have their point of departure in perivascular lesions of the meninges. Their site of election is either the cortical region, the deeper ganglionic masses or the white matter. They take at times the form of small foci along the lymphatic sheaths of the blood vessels efferent from the pia mater, or of caseated nodules surrounded by a red hemorrhagic zone. Now and then too, large isolated or solitary tubercles (*tubercules solitaires*) develop and reveal their presence by particular symptoms associated with their localization. Thus tubercles have been pointed out in the cerebral peduncles (Raviart), in the pons (d'Astros and Hawthorn), and in the optic thalamus (Demange and Spillmann, Linguet), etc.

These solitary tubercles are said to be more frequent in the young, and particularly in children from 3 to 10 years old. They may vary in size from that of a pea to that of an egg, and are ordinarily composed of dense caseous matter with a surrounding zone of gray granulation. Their centers become soft and, if evacuated, leave a veritable cavity in their place.

In the spinal cord the tubercles are likewise found either conglomerate or isolated. If large enough they set up a more or less extensive degeneration of the nervous tissue of the columns, interrupt the continuity of the medullary tracts and thus cause functional disturbances which are ordinarily very serious.

Infection of the cord may also appear in the guise of a meningomyelitis. The tubercles then develop along the vessels, in the lymphatic sheaths which surround them. They lead to degenerations in the bundles of nerve fibres, disintegrate the myelin sheaths and cause swelling of the axis cylinders.

It is very difficult to reproduce these types of lesions experimentally. They are rare clinically and are encountered only occasionally at autopsy.

CHAPTER XV

TUBERCULOUS INFECTION OF THE LIVER, SPLEEN, KIDNEYS AND INTESTINE

A. TUBERCULOUS INFECTION OF THE LIVER

Localizations of tuberculous infection in the liver are very frequent at all ages. Except in certain quite exceptional cases of direct contamination through the lymphatic or umbilical blood vessels in the new-born, they do not appear to be primary infections. (A case of this sort was reported by Sabouraud). As usually observed, they are secondary to other localizations, peritoneal, intestinal, glandular, glandular-pulmonary or pleural for example, and it is known today that, contrary to long accepted opinion, tuberculosis of the liver is found constantly in phthisis, and almost constantly in the chronic tuberculous, provided pains are taken at autopsy to examine this organ with the microscope or even with the magnifying glass (Arnold,[1] Brissaud and Toupet[2]).

For the most part tuberculous lesions of the liver remain very small and latent; moreover they do not reveal themselves by any striking symptoms. They take origin in the lymphatics of the connective tissue walls of the bile ducts or blood vessels and there develop, either forming typical tuberculous nodules with giant cells or becoming arrested in the lymphoid stage to spread themselves widely in the portal spaces. They then set up a fatty or amyloid degeneration of the liver cells and result at times in a necrotic process with the formation of larger or smaller islands of steatosed and caseous tissue.

Subsequently there is a variety of clinical forms of the disease analogous to those found in the lung, but more difficult to individualize since they often pass from one form to another in the same patient. Nevertheless one may, like Gougerot,[3] group them in the following

[1] Virchow's Arch., 1880, **132**, 502.

[2] J. de Verneuil, 1897, fasc. I.

[3] Rev. de la tuberc., 1906, **3**, 472.

order: degenerations and atrophies, tubercles, cirrhoses, forms of parenchymatous hepatitis.

1. Degenerations and atrophies

To these are due the symptoms of hepatic insufficiency so frequently observed in tuberculous patients. The fatty or amyloid degenerations are not specific; they occur in a large number of infectious processes. They are characterized at first by a cloudy swelling of the cells, the result of infiltration of the protoplasm by fat globules and granules of pigment, afterward by changes in the nuclei which become vesicular. Areas of hepatic cells loaded with bile pigment are marked off by ramifications of the hepatic veins and give to the organ the appearance designated as *nutmeg liver:* this is the red atrophy of Sabourin.[4] Or else these areas become surrounded with connective tissue cells which enlarge and press together so that the hepatic cell protoplasm atrophies and then disappears: the condition is then one of *atrophic cirrhosis.*

These lesions appear due to the direct fatty-degenerative action of the tubercle bacillus (Hanot and Lauth, Péron) or of the fats which enter into its composition (*éthéro-bacilline* of Auclair, according to Ribadeau-Dumas). It seems impossible to reproduce them with tuberculin alone (Carrière, L. Bernard and Salomon).

In amyloid degeneration the perilobular and perivascular connective tissue fibres become swollen and the capillaries are choked off and destroyed little by little in a coagulated hyalin mass. The latter may be regarded as the outcome of the intra- or extra-cellular precipitation phenomena induced by the action of tuberculin on the protoplasmic albumins which are more or less rich in tuberculous antibodies.

2. Hepatic tubercles

True tubercles in all their variety occur in the liver as in other organs, from the microscopic nodules of acute miliary tuberculosis to tubercles the size of a nut.

There is nothing peculiar about their composition, and their lymphoid origin is always the same. According to circumstances they undergo the usual processes of caseation or sclerosis. If they progress

[4] Arch. de physiol., 1884, **2**, 47.

to softening they form true biliary cavities or large cold abscesses (Lannelongue) with grumous, greenish pus, and occasionally ulcerate through the diaphragm and into the lung and evacuate themselves as vomica.

The tuberculous process may invade the larger and smaller bile ducts to a greater or lesser extent, thus causing a true tuberculous suppurative angiocholitis. The tuberculous nodules are then softened, being macerated by the very alkaline bile with which they are infiltrated. Bacilli are usually rare. It appears that this sort of infection of the bile ducts cannot occur except by the descending path, at least experimentally according to Sergent,[5] and it is supposed to result from tubercle bacilli being brought from intestinal ulcerations by the portal vein.

3. Tuberculous cirrhoses

The processes of interstitial sclerosis, perilobular and perivascular, resulting from diffuse tuberculous infiltration of the liver, manifest themselves in the various forms of cirrhosis which clinicians differentiate according to the predominant type of lesion: tuberculous fatty hypertrophic hepatitis of Hanot-Gilbert, simple hypertrophic cirrhosis of Hanot-Gilbert, chronic hypertrophic cirrhosis, forms with or without ascites, forms with protracted icterus or successive attacks of icterus, atrophic liver, cardio-tuberculous cirrhosis through chronic passive congestion (*cirrhose sus-hépatique par stase cardiaque*) of Hutinel, etc. All of these varieties depend, from the pathologico-anatomic point of view, on the predominating type of cellular lesion.

4. Tuberculous parenchymatous hepatitis

This may be nodular or diffuse. If nodular, it is according to Sabourin, secondary to the retention of bile; but, according to Kelsch and Kiener, and Macaigne, it is simply the result of an inflammatory reaction on the part of the liver against tuberculous infection. It may be accompanied by a fatty or amyloid degeneration. Tubercles are not constant. When present they are small and enclosed in sclerotic tissue.

If diffuse, parenchymatous hepatitis (Gilbert and Surmont) takes the form histologically of a very active proliferation of cells

[5] Compt. rend. Soc. de biol., 1895, 47, 336; 351:—Presse Méd., 1896, i, 177.

in the hepatic columns, of an infiltration of fat into the portal spaces, and of the atrophy of a large number of normal hepatic cells which are replaced by small cell elements poor in protoplasm and with nuclei which stain deeply with carmine and hematoxylin.

All of these varied forms of tuberculous infection of the liver are due to differences in the manner in which the organ reacts to infection by the bacillus, and these differences depend upon the intensity of pre-existing infection, the susceptibility or resistance of the subject, and chiefly probably upon the nature of the reactions, within the interior itself of the hepatic cells, between the albuminoid protoplasmic substances and the tuberculous toxins.

B. TUBERCULOUS INFECTION OF THE SPLEEN

In the child, even more than in the adult, all infectious processes, and particularly tubercle bacillus infection, react upon the spleen with great intensity by reason of its function of producing lymphocytes and mono- and polynuclear leucocytes, and also because of its connective tissue and lymphatic structure.

It is not surprising therefore that, in acute miliary tuberculosis, the spleen is the seat of confluent miliary lesions and that, in almost all grave forms of tuberculosis, it is found invaded to a greater or lesser degree by the tuberculous process. But it is only rarely that the latter is very apparent. The tubercles remain in general very small, hidden in the splenic pulp and only barely visible unless sought with care, and consequently as a rule escaping unobserved at autopsy.

These tubercles make their appearance first in the lymphatic glands of the hilus whence they extend to the interior of the organ and frequently also to its capsule. Masses of caseous nodules, whose size may vary from that of a pea to that of a nut, are rare. It is exceptional for them to reach the stage of softening. The so-called *lenticular* tubercles are those most commonly observed. They are enveloped in thick connective tissue and tend to undergo fibrous degeneration. They occur first in the *Malpighian bodies* and, from there, extend by the perivascular lymph channels, to be found as a rule clustered about a small vessel, like a bunch of grapes.

Tuberculosis of the spleen, except in the acute miliary form, always remains latent and manifests itself only by a little splenic enlargement.

The spleen certainly plays a very important protective rôle against tuberculous infection. Splenectomy, in consequence, favors infection in animals, and the experiments of F. Arloing[6] showed that, if the spleen was removed *before* intravenous infection, tuberculous lesions progressed much more rapidly to caseation than if the spleen was removed *after* infection.

C. TUBERCULOUS INFECTION OF THE KIDNEYS

Tuberculous infection of the kidneys, not very frequent at best, is almost never primary. It originates almost uniformly in a blood infection, and its forms vary in their mode of evolution according as the individual has or has not acquired, from the fact of his having recent or old lesions, that special intolerance to the bacillus which is disclosed by the *phenomenon of Koch* (*see Chapter XXXIX*) and which characterizes immunity against tuberculosis.

Renal tuberculosis is most frequent between the ages of 12 and 30 years.

Children are more often affected than adults. J. Hallé[7] reports that Dickinson, in 300 autopsies of adults, found tubercles of the kidney only 17 times, whereas, in 300 children, he detected 49 cases of renal tuberculosis. Moreover Rilliet and Barthez found 49 children with tubercles of the kidney among 312 autopsies of tuberculous cases. Oscar Muller[8] puts the frequency of renal tuberculosis in tuberculous children at 23 per cent. Schwer estimates that the proportion is still higher, since he found tubercles of the kidney 83 times in 123 autopsies. M. Letulle, Tamayo and A. Jousset share this opinion. According to them, a fifth or even a quarter of phthisical cases have diseased kidneys.

The very different clinical forms affected by renal tuberculosis may be divided into two pathologico-anatomic types which are:

1. Renal tuberculosis properly speaking with more or less extensive miliary lesions.
2. Tuberculous nephritis with non-miliary lesions.

[6] Compt. rend. Soc. de biol., 1904, **57**, 524.
[7] In *Traité des maladies de l'enfance*, by Marfan, Vol. III, p. 345.
[8] München. med. Wchnschr., 1889, **36**, 875.

I

Renal tuberculosis with miliary lesions results from the development of tubercles in the capillaries of the kidney glomeruli. Experimentally it may be produced very easily in animals, as has been done by A. Borrel,[9] who injects suspensions of bacilli directly into the aorta of a rabbit, sending the injection as far as the aortic arch by means of a blunt cannula introduced into the carotid, and at the same time compressing the other carotid in order to prevent the possible return of the bacilli by that path. In this way kidney infection is almost certain whereas by intravenous injection one is successful only very rarely.

Léon Bernard and Salomon[10] also succeeded in obtaining very interesting results in the rabbit by inoculating bacilli into the left ventricle of the heart, and in the dog by inoculating them into the femoral artery.

Under these conditions, which have the objectionable feature of having brought about *primary* infection of the organ in animals *free from all previous tuberculous infection*, it was found that the bacilli become arrested in the glomerular capillaries, are ingested by the polynuclears, and then provoke an influx of mononuclears which in their turn ingest the polynuclears and bacilli. The mononuclears become degenerated and beget the characteristic early miliary tubercles (*follicules*). These are located exclusively in the substance of the cortex. A few days later miliary tuberculosis is seen to appear. Lymphatic cells have accumulated in the interstitial spaces of the pyramids and are there transformed into epithelioid cells, whence come new granulomata, less rich in bacilli than the first and developing throughout the thickness of the medullary substance.

Spontaneous infection in man, does not seem to differ in its pathogenic mechanism, from that thus realized experimentally. It occurs only rarely as a miliary tuberculosis, since questions of dosage of virus and of individual resistance modify the process.

This miliary form is scarcely ever encountered save in acute generalized miliary disease. Only exceptionally is it localized exclusively in the kidneys, and is then very discrete. A few cases have been described by Potain, Chauffard, Castaigne, and Albarran.

[9] Ann. de l'Inst. Pasteur, 1894, **8**, 65.

[10] Compt. rend. Soc. de biol., 1904, **57**, 526; 1905, **58**, 71; 94; 1906, **61**, 414:—J. de physiol. et de path. gén., 1905, **7**, 303; 1906, **8**, 673.

Tuberculous infiltration of the kidney (ulcero-caseous, fibro-caseous, chronic or surgical forms) is much more common. It presents itself in the most varied guises. Noel Hallé[11] distinguishes two anatomical forms differentiated by the site, appearance and evolution of the lesions: *parenchymatous tuberculosis primarily closed* and *pelvic tuberculosis primarily open.*

In the first, lesions of the kidney lobules, and particularly those at the poles, are circumscribed and encysted. Their tendency is to fibrous degeneration, to obliteration by retraction, and to cicatrization. They lead to the *partial or total exclusion* of the diseased kidney.

In the second, the lesions, which are at first extra-renal, invade the kidney secondarily and destroy it by progressively ulcerating outward and soon forming one or more cavities opening into the kidney pelvis. Tuberculous pyonephrosis results. There is but little tendency to become encysted and cicatrized, and they are frequently infected secondarily with pyogenic microörganisms. The presence of the latter hastens and aggravates the process of destructive ulceration.

Ordinarily the kidney is enlarged, with prominent bosses, and sown with little or big tuberculous masses which may be caseated or may enter into the formation of large cold abscesses. The latter are located particularly in the cortex and may discharge into a calix (whence pyelonephritis and tuberculous pyuria). If the ureter is included in the process, it becomes at times no longer patent and there is observed a distention and afterwards the progressive purulent softening of the kidney (tuberculous hydronephrosis and massive degeneration) which becomes filled with thick cheesy pus, resembling putty.

The softening of the caseous masses in the kidney may be of any degree; from the formation of small cavities to the progressive elimination of the calices and pyramids, leaving the organ excavated by great cavities in almost immediate contact with the membrane of the capsule (*see Plate IX, 2*).

Where pyuria exists, the bladder but very rarely escapes involvement. Granulations appear on its internal surface about the ureter which discharges the bacilli, and cystitis makes its appearance.

[11] J. méd. français, July 15, 1914.

It often happens moreover that the glands of the renal hilus and lumbar chain are invaded and become more or less caseous.

Renal tuberculosis is fairly frequent and is one of the forms of latent infection which longest escapes the clinician's attention. That it is *usually* capable of cure is a fact no longer open to doubt, according to the work of J. Castaigne, A. Lavenant and E. Benazet, Legueu, Papin and Verliac.[12] Healing may occur spontaneously by sclerosis, or more rarly, by calcification. Often enough, at autopsy, kidneys show the single or multiple scars of collapsed cavities, retracted and composed of fibrous tissue which is relatively dense and much pigmented.

II

Clinicians accept that certain forms of nephritis in the tuberculous are not dependent upon the presence of the bacillus in the renal tissue, but result from a true intoxication by products derived from the microörganism.

In support of this idea they cite the opinion of such investigators as Grancher, H. Martin and Ledoux-Lebard, S. Arloing, Rodet and J. Courmont, Léon Bernard and Salomon,[13] and others, who have observed renal changes, from simple congestion to atrophic sclerosis, ensue after inoculation of attenuated bacilli or tuberculin, or the éthéro-bacilline of Auclair.

The facts adduced however do not appear altogether convincing. Tuberculin in small doses in tuberculous subjects, and in large doses in healthy animals undoubtedly exercises a congestive action upon the kidney and *particularly upon the suprarenal capsule*; nevertheless, from an anatomical standpoint, the changes brought about are not comparable to those found in nephritis. This was clearly shown by the experiments of A. Jousset and those of L. Bernard and Salomon. By systematically examining serial sections in parenchymatous nephritis and by inoculating fragments of tissue, A. Jousset[14] was always able to demonstrate more or less typical incipient tubercles (*follicules*). In such cases, the infections are less intense or are produced by less virulent bacilli.

[12] J. méd. français, July 15, 1914.

[13] Compt. rend. Soc. de biol., 1903, **55**, 1306;—J. de physiol. et de path. gén., 1904, **6**, 884.

[14] Thèse, Paris, 1909.

D. TUBERCULOSIS OF THE SUPRARENAL CAPSULES

The suprarenal capsules are seldom affected with miliary tuberculosis, except when the infection is generalized. Chronic infection in the form of isolated tubercles, having their point of departure in an obliteration of the central vein of the gland, is much more frequent, as M. Letulle has shown. Now and then an inflammation of the peri-suprarenal tissue develops with caseation of the underlying gland tissue, forming cavities or undergoing hyalin or calcareous degeneration here and there; or a true cold abscess may be produced.

There is also a variety of lesion without tubercles, to which Sezary,[15] then Poncet and Leriche,[16] and Milhit called attention, and which is characterized by sclerotic transformation of the glands. The covering membrane strips off with difficulty and the gland capillaries are compressed in very dense connective tissue strands, so that small, congested, nodular-appearing islands are formed in the midst of the sclerotic tissue. The latter extends even to the medullary portion where the cells shrivel and atrophy.

In these lesions neither tubercles nor bacilli are to be found. Loeper and Oppenheim believe that they have produced them experimentally with the éthéro-and chloroformo-bacilline of Auclair. Perhaps they result from a specific toxic action of the protoplasmic poisons of the tubercle bacillus on the gland. Injections of tuberculin in tuberculous animals always produce altogether analogous effects: intense congestion and considerable swelling of the suprarenal when the dose of tuberculin approaches the lethal amount, sclerotic transformation when the tuberculin is injected in small and repeated doses over a long period. In the latter case the resulting suprarenal insufficiency often leads to death of the animals.

To these more or less deep lesions of the suprarenal, which are observed quite often in the chronic tuberculous, clinicians attribute certain symptoms, such as asthenia, sensation of cold, tendency to collapse, hypotension, melanoderma (Addison's disease), unusually ready pigmentation on exposure to the sun; perhaps also arteriosclerosis and atheroma (Poncet and Leriche).

[15] Arch. de méd. expér., 1904, **16**, 521.
[16] Bull. Acad. méd., 1911, **65**, 711.

E. PATHOLOGICO-ANATOMIC CHARACTERISTICS OF TUBERCULOUS LESIONS OF THE INTESTINE

Although the intestine, over most of its length, possesses an absorbing surface which is permeable to minute particles, and consequently by bacilli, as we have already seen (*Chapter X*), *primary* tuberculous lesions exist there very seldom, even in nurslings. On the other hand, the lesions of *secondary* infection, produced by way of the blood stream, are fairly frequently observed in the child, are common in phthisical adults and may be reproduced experimentally in animals by simple intravenous injection (Loeper and Esmonet, Ch. Richet, Jr.[17]).

The rarity of primary tuberculosis of the intestine is an argument still used by a certain number of clinicians against the conception of the digestive tract as the leading factor in the absorption of virulent elements in tuberculous infection. These clinicians, with too much respect for dogma, are unwilling to renounce their faith in the *law* of Cohnheim, despite experimental proofs.

Meanwhile we know, through numerous works of which the majority are cited in Chapter X, that the tubercle bacillus, like many other inert or bacterial solid particles, penetrates through the mucosa of the intestine without leaving the slightest trace of its passage and without setting up, *in loco*, the slightest lesion (Chauveau, Dobroklowski, Von Behring and Römer, Calmette and Guérin, Orth and L. Rabinowitsch, and others.).

It is only when tuberculous infection is brought about by a massive dose that one or more inoculation lesions (*chancres d'inoculation*) may develop in the intestinal mucosa, and they then locate themselves in the lymphoid glands, *Peyer's* patches, or in the solitary follicles, to extend later by the lymphatic channels into other glandular groups (principally the mesenteric, omental and periportal). This process is seen at times in infants nursed by phthisical mothers or fed with raw milk from cows infected with mammary tuberculosis. Although not exceptional, it is relatively rare.

Usually the tubercles of the intestine are formed in the lymphatic network beneath the mucosa, as the result of the obliteration of some capillary blood vessel through a lesion within or perivascular. Now and then, following very massive infections, they take the

[17] Thèse, Paris, 1912.

form of a genuine eruption of rather large and confluent nodules which project under the peritoneal covering of the intestine and later elevate the mucosa to ulcerate at its surface (*see Plate VIII, 1, 2, and 3*).

It is much more common to find, in young subjects who have caseated tubercles in the mesenteric or tracheo-bronchial glands, tuberculous ulcers of the intestine which are formed only secondarily and through successive invasions. Their quite peculiar appearance is evidence of their vascular origin. They are *lesions of auto-reinfection.* They are commonly found also in adults, and almost constantly (9 out of 10 times) in cases of chronic phthisis, according to Frerichs, Höning, Herscheimer, and Weigert. In 215 autopsies of phthisical cases, Louis noted them 174 times.

Their location of choice is the middle portion of the small intestine (jejunum), then the portions of the ileum and caecum near the vermiform appendix, that is to say where the sub-mucous lymphatic system is most highly developed. The large intestine meanwhile is not spared, but large ulcerations are there less frequent.

At the site of these lesions the intestinal wall is edematous, at times thickened, again much thinned and easily ruptured. All about is a red or violet-colored inflammatory zone.

Tuberculous ulcers assume four principal types:

1. The *lenticular* type, in which the sub-mucous tubercles stand out in rather large number, but isolated, forming small tumors which are flattened at first and later project until they resemble small figs attached to the wall of the digestive tract. This is the type most frequently encountered in children (*Plate VIII, 1*).

2. The *annular* type, characterized by true ring-formed ulcerations which cut the mucosa transversely. This form usually follows obliterating endarteritis. It happens at times that several ulcerations are found succeeding one another at almost equal intervals over a section of intestine. Their margins project but little and are full of small tuberculous granulations. The floor is of a slate-like or reddish color; they lay bare the submucosa (*Plate VIII, 2*).

3. The *longitudinal* type, which develops by preference in *Peyer's* patches and erodes their whole extent more or less deeply, to spread much further afterward, at times over 8 and 10 centimeters of length (*Plate VIII, 3*).

4. What one may call the *irregular* type, since it tends to all sorts of conformations: rounded, linear, sinuous or serpiginous. This is the form most frequently encountered at the level of the caecum and transverse colon. The ulcers composing it have prominent, granular and loosened margins. Their floor is reddish brown, marbled in appearance and, in the fresh state, full of caseous matter containing bacilli in abundance.

The lymphatic glands of the mesentery, and the other gland groups further along the course of lymph flow, always participate with varying degrees of intensity in the tuberculous process which invades the zone of intestine to which they correspond.

These ulcerative lesions may progress to perforation of the intestine, which is rare in children, to perforation of a large mesenteric vessel, or to apparent healing by sclerosis. It is then what is called the *stenosing* form, common in adults.

This stenosing form produces cicatricial constrictions which are located most often in the region of the caecum. The constrictions may be multiple, accompanied by considerable thickening of the mucosa, and may reduce the lumen of the gut to the point of causing serious functional disturbance. Fortunately, surgical intervention may relieve the latter.

Histological study of the changes in the tuberculous intestine has been made by Wesener,[18] Höning,[19] Spillmann,[20] Girode,[21] Tchistovitch,[22] and Patel.[23] It was found that, as regards the genesis and development of the lesions, the epithelial and gland elements are affected only secondarily. The essential rôle belongs to the lymphatic elements.

Experimentally, Von Baumgarten[24] found that, in animals infected by the ingestion of tuberculous matter, the nodules appear first in the closed follicles of the intestine, and that they do not invade the neighboring tissues until later.

It is very probable that infection of the closed follicles is brought about most often by way of the blood stream. This view is upheld

[18] Deutsch. Arch. f. klin. Med., 1884, **34**, 583.

[19] Inaug.-Diss., Bonn, 1885.

[20] Thèse, Paris, 1878.

[21] Thèse, Paris, 1888.

[22] Ann. de l'Inst. Pasteur, 1889, **3**, 209.

[23] Thèse, Lyon, 1901/02.

[24] Berl. klin. Wchnschr., 1902, **39**, 643.

today by Loeper[25] who, with Esmonet, reproduced true intestinal tuberculomata by the injection of bacilli into the intestinal arteries of a dog. Similar lesions are now and then found in guinea pigs infected by the blood stream, or even subcutaneously. The most common localization is in the second portion of the ileum, which is the richest in blood vessels and lymphoid tissue.

The diarrheal stools of those who have intestinal ulcerations and tuberculous enteritis contain bacilli in larger or smaller number according to the extent of the lesions. It is not always easy to discover them since they are scattered through an enormous mass of material. They can be detected by one of the antiformin methods of which the technique was described in Chapter I (C), and as already stated in Chapter II, their nature must be determined by inoculation into the guinea pig, in order to eliminate the acid-fast saprophytic bacilli.

But in a case of phthisis where bacilli are abundant in the sputum and, as always happens, a part of them are swallowed, it becomes difficult to determine whether the bacterial elements are derived from the sputum or the intestinal lesion. Nevertheless, if *masses of tubercle bacilli* are found in the feces on direct examination of slides stained with *Ziehl,* it is highly probable that they have originated in ulcerations of the intestinal mucosa and not in the bronchial secretions which are mixed with food and drink in the stomach and throughout the length of the digestive tract.

[25] *Leçons de pathologie digestif.* 2nd ser., 1912. p. 210.

CHAPTER XVI

LOCALIZATIONS OF TUBERCLE BACILLI IN BONES AND JOINTS

Bone tuberculosis is observed at all ages. In the nursing infant however and in the aged it is exceptional. It is particularly from the third to the fifteenth year that it becomes frequent.

In studying the previous history of such cases, it is almost always found that localization of the tuberculosis in bone or joint is the outcome of a general disease; it occurs after a blood infection ordinarily unrecognized and called grippe, atypical typhoid fever, or protracted gastric disturbance, etc. (Calvé).[1]

It is seen to occur generally in subjects who have lived in contact with the tuberculous; but it makes its appearance now and then in others who do not seem to have been exposed to frequent contamination, but have been fed by chance with suspected milk.

In England, Nathan Raw (of Liverpool) thought that he had succeeded in demonstrating that the majority of cases of bone or joint tuberculosis, at least in children, are due to milk infection and that the causal bacilli are almost always of bovine origin. This opinion is shared by John Fraser[2] (of Edinburgh) who collected the histories of 70 cases. There were 52 of these who presented no history of tuberculosis nor of living with the tuberculous. In the latter group, bovine bacilli were found in 43, and human bacilli in but 9, or 17 per cent.

A. PRINCIPAL FORMS OF BONE TUBERCULOSIS; THEIR GENESIS

Bone tuberculosis assumes a great variety of forms. Most commonly it attacks the spongy bones, the red marrow and the neighborhood of the cartilaginous surfaces between epiphysis and diaphysis, or the periosteum.

Tuberculosis locating primarily in the diaphysis almost always begins as a focus of medullitis, which may be at any point of the

[1] Bull. Soc. d'étude scient. sur la tuberc., 1912, May 9.

[2] J. Exper. Med., 1912, **16**, 432.

canal, but which, most frequently, as in osteomyelitis, begins at the level of the bulb. It may invade the entire marrow or remain localized in the periosteum and erode only the subjacent bony tissue.

In that form of tuberculosis of the diaphysis called *spina ventosa*, the bone, softened at the centre, thickens at its periphery and becomes enlarged. This variety is particularly frequent in young children, especially in boys, and is commonly attended by glandular or other joint localizations.

Nélaton listed the bones in the relative order of frequency of tuberculous localization as follows:

1. Vertebrae;
2. Tibia, femur, humerus;
3. Phalanges, metatarsals and metacarpals;
4. Short bones of the tarsus and carpus;
5. Petrous portion of the temporal bone.

Volkmann in 1888 described perforating tuberculosis of the vault of cranium.

Young[3] studied 1000 cases of bone tuberculosis and lists them in the following order:

REGION	NUMBER OF CASES	PERCENTAGE
1. Vertebrae	416	41.6
2. Hip joint	421	42.1
3. Knee	103	10.3
4. Astragalus	33	3.3
5. Shoulder	2	0.2
6. Elbow	17	1.7
7. Carpal joints	8	0.8

The pathogenesis of tuberculous lesions of bones or joints is the same as that of other organs. They result always from the lodgement in a lymphatic vessel, or more rarely in a blood capillary, of a polynuclear leucocyte which contains phagocyted bacilli. This leucocyte becomes the centre of a beginning tubercle (*follicule*).

The development of this intra- or perivascular tubercle and the extension of the resulting lesion are from then on governed by varions factors whose rôle we already know; number and virulence of infecting bacilli, degree of vascularization of the tissue at the site of the begin-

[3] *Orthopedic Surgery*, Philadelphia, 1906.

ning tubercle, age and resistance of the subject, and grade of immunity conferred by previous glandular tuberculosis.

The seriousness of the affection is subject to the same factors. If the condition is one of *primary infection*, or of a *reinfection very closely following the primary*, and is produced by bacilli newly derived from another human being, the disease tends to a rapidly extensive form, quite different from that observed in individuals partially immune by virtue of an old concealed tuberculosis, and in whom the disease evolves extremely slowly and with a natural tendency to healing.

On many occasions attention has been called to the influence of traumatism, particularly during the period of skeletal growth, as prompting the localization of tuberculosis in bones or joints. Some time ago, Max Schüller tried to demonstrate this and performed certain experiments about which there was some commotion. After having infected his animals, he submitted them to various traumatisms and observed tuberculous osteo-arthritis develop at the points of injury. Non-infected animals, on the other hand, although subjected to similar traumatism, exhibited only a hemarthrosis which cured spontaneously.

These experiments of Schüller were later repeated with more perfect technique and quite different results by Lannelongue and Achard,[4] by Friedrich,[5] Honsell,[6] Fr. von Friedlander, and others, so that at the present time surgeons are rather of the opinion that the rôle of traumatisms in the generation of bone and joint tuberculoses is relatively limited.

In 1892, Pawlowsky,[7] in Metchnikoff's laboratory at the Pasteur Institute, attempted to follow in the guinea pig the evolution of articular lesions attending the injection of pure cultures of bacilli into the knee joint. The latter he excised after various intervals, 6 hours, 1, 2, 3, 4, 6, 8, and 10 days, and after 2, 3, 4, 5, 6, and 8 weeks. Microscopic examination of sections showed that hyperemia of the cartilage does not become apparent until about the fourth day. About the sixth day the joint is swollen and the synovial membrane takes on a leathery appearance; the inguinal glands are enlarged.

[4] Internat. Congr. on Tuberc., Berl., 1899.

[5] München. med. Wchnschr., 1899, **46**, 1313.

[6] Ibid., 1900, **47**, 1831.

[7] Ann. de l'Inst. Pasteur, 1892, **6**, 116.

After three weeks the joint is often full of pus and soft granulations. A little later a rather profuse suppuration is established, and the articular surfaces are covered with fungous formation.

Beginning with 12 hours, in stained preparations, bacilli may be demonstrated within the leucocytes and passing through the synovial endothelium into the lymphatic interstices of the periarticular connective tissue.

In Metchnikoff's laboratory, in 1904, Petroff[8] (of Petrograd) carried out a more complete study than had been made up to that time of the influence of trophic and vasomotor disturbances, produced for example by sectioning a nerve trunk or an important blood vessel, upon the localization of tubercle bacillus infection in the bones.

Three series of experiments were performed on rabbits. The first two consisted respectively in section of the abdominal sympathetic and of the sciatic; the third in ligation of the femoral vein. A suspension of tubercle bacilli was afterward injected into the ear vein.

The results were that, in 5 cases where tuberculous foci could be discovered in the hind legs, they were found symmetrically involving the intact as well as the operated side.

B. INFLUENCE OF MIXED INFECTIONS IN TUBERCULOSIS OF THE BONES

Petroff also sought to define the rôle of mixed infections in bone tuberculosis. It was known indeed that the prognosis for tuberculous foci differs according as they are open or closed, and that although closed foci generally contain only tubercle bacilli, they are now and then infected with a streptococcus. (V. Brunn).

In pus from 45 cases with open cold abscesses in Lannelongue's service, Petroff[9] found the staphylococcus in 25 instances, the streptococcus in 18 instances, pseudo-diphtheria bacilli 8 times, *Bacillus pyocyaneus* 4 times, *Micrococcus tetragenus* twice, *Bacillus coli* once, and several other undetermined varieties of saprophytes.

On the other hand, of 57 cases in which the cold abscesses were closed, culture of the pus in 49 instances gave no growth. From the 8 other cases, cultures of *Staphylococcus albus* were obtained 3 times, a streptococcus twice, and other saprophytes 3 times, all of which were probably contaminations due to the fact that the abscesses were adjacent to fistulous tracts.

[8] Ann. de l'Inst. Pasteur, 1904, **18**, 590.

[9] Centralbl. f. Bakt. Ref., 1904. **34**, 54.

By artificially infecting the knee joint in rabbits, first with the tubercle bacilli and afterward with the pyogenic bacteria obtained from cultures of human tuberculous products, Petroff satisfied himself that the foci of granulation and destruction were always considerably more extensive in the secondarily infected points than in those which remained purely tuberculous. The cartilages and bone epiphyses forming part of the joint were involved in the destructive process in cases of double infection, whereas in the joints infected solely with tuberculosis only the synovial membranes and the intra-articular ligaments, that is to say the least resistant tissues,—were diseased. It was established moreover that secondary infection hastens noticeably the general spread of the original infection.

C. PATHOLOGICO-ANATOMIC CHARACTERISTICS OF BONE AND JOINT TUBERCULOSIS

The pathogenesis of bone tuberculosis has been, from the histological point of view, the object of most careful researches on the part of John Fraser[10] who was able to study 80 different types of lesions and to follow the infecting process step by step.

According to this investigator, all bone tuberculosis begins with an osteomyelitis, and the latter originates in a minute tubercle which is formed in the marrow and which in the immense majority of cases is perivascular and therefore of lymphatic origin. The initial lesion is a proliferative inflammation of the medulla going on to the development of *fungosities*. The latter are composed of collections of greyish granulations with connective tissue fibers buried in the mass of leucocytes. Little by little the granulations become more or less confluent caseous tubercles, or else, under favorable circumstances, they atrophy and give place to cicatricial fibrous tissue.

When the lesions extend to the medullary tissue, the bony trabecular system is rapidly involved. Sometimes layers of osteoblasts accumulate around the tubercles and there press together in successive deposits, to constitute *condensing osteitis*. Again the bony partitions undergo a true lacunar corrosion, the calcareous cement disintegrates, the bony substance is transformed into fibrous tissue, and the bone corpuscles disappear through fatty degeneration (Ranvier). This is the *rarefying osteitis*. The two processes ordinarily coexist in different parts of one and the same lesion.

[10] J. Path. and Bact., 1912, **17**, 254; J. Exper. Med., 1913, **17**, 362.

In the midst of the fungous or caseous masses small fragments or sequestrated particles (*séquestres parcellaires*) of the bony trabecular system are usually encountered. One may also come across larger bony masses cut off from all blood supply and more or less altered by the tuberculous process; these represent the *necrosis sequestra* of Ollier.

Tuberculous lesions in bone tissue evolve slowly as a rule. At times however there is rapid invasion of a bone which becomes the seat of confluent lesions quite comparable to miliary tuberculosis of the lung (*acute tuberculous osteitis*).

In the chronic forms, which are by far the most common, there are distinguished: *encysted tuberculous lesions*, *tuberculous infiltration*, *caries* and *spina ventosa*.

Encysted tubercles result from the development, in the heart of the medullary tissue, of a few tuberculous granulations which have formed a caseous nodule. They represent true abscesses within the bone.

Tuberculous infiltration and caries end in a more or less extensive destruction of the trabecular tissue, with or without the formation of sequestra.

If the process of infiltration invades the periosteum, either principally or subordinately, a simple local lesion may be produced, or granular tissue may form over a more or less extensive surface of bone which is resorbed. A *peripheral caries* is the result.

Tuberculous foci of the periosteum generally caseate sooner or later; when they do not heal they soften little by little and, as in the bone marrow, caseous nodules are gradually formed, which are surrounded by granulations and hardened connective tissue. In other cases larger or smaller *cold abscesses* make their appearance, their enveloping membrane made up of tuberculous nodules and connective tissue. These abscesses steadily enlarge through the accumulation within them of caseous degeneration products from their walls.

A cold abscess may migrate from its point of origin into the neighboring organs and thus form an "*abcès par congestion*" or migratory abscess. This is often observed following tuberculous caries of vertebrae (*Pott's disease*); the abscess then descends along the psoas muscle as far as the iliac bone and Poupart's ligament.

In *spina ventosa*, which is observed only in children and which affects exclusively the long bones of the hands and feet, the medullary

tissue disappears, being replaced by a yellow fungous mass which sometimes forms a hernia through the periosteal sheath. The bone walls are thinned out and swollen over the whole extent of the diaphysis. At times the epiphyses even are affected and the result is a simple or double suppurative arthritis.

In tuberculosis of the joints, the most common form is the *white swelling* (*synovite fongueuse or tumeur blanche*), characterized by the development of diffuse buddings in the synovial membrane. These vegetations are limited as to blood supply, and are of a whitish transparency, like the flesh of an eel, as Panas remarked. In a few cases they are well supplied with vessels and then take the color of wine dregs. At times, they develop slowly, again very rapidly according to the intensity of the causal infection. They are composed of embryonal tissue containing tuberculous nodules, and tend to spread peripherally, while the center undergoes fatty degeneration.

In cases of white swelling, the cartilage is always affected, but secondarily. It may be infiltrated with tubercles, or detached and macerated, or finally destroyed. It always constitutes a barrier which for a certain time protects the subjacent bony tissue against invasion.

It is a well established fact that the majority of forms of bone tuberculosis heal with relative ease in children, while the prognosis is in general much more grave in adults. There are several reasons for this. Probably the most important is that, in growing individuals, the defensive cellular reactions are more intense and the reparative processes in bony or fibrous tissues proceed much more energetically.

Another reason is that in children of 3 to 10 years of age, who most often develop the bone and joint localizations, the latter in a great many cases are the echo, so to speak, more or less loud, of a primary glandular infection of recent date; a primary infection produced by an attenuated bacillus of bovine or human origin.

In the adult, on the contrary, the *infections are almost always secondary and to some extent recurrent*, or they are *auto-reinfections* in a individual weakened by wasting disease.

The chief element of danger in both cases is tuberculous infiltration of the viscera. The patients then die of pulmonary tuberculosis or meningitis. According to Billroth and Koenig this is the fate of about 16 per cent of patients treated for white swellings.

CHAPTER XVII

CUTANEOUS LOCALIZATIONS IN TUBERCULOUS INFECTION

Their Pathologico-Anatomic Characteristics

Since tuberculosis is essentially a disease of lymphatic tissues and organs, it is rather astonishing that the skin, or rather the dermis, above all in those parts of the body particularly rich in lymphatic vessels, does not more frequently provide favorable conditions, from childhood, for the development *in situ* of tuberculous infection.

This indeed, is not the case, since localization of tuberculosis in the skin is relatively rare. Children and women with their more delicate skin, are more subject to it than men.

Tuberculous infection of the skin assumes forms differing greatly from one another, without its being possible at the present time to grasp the causes of these diversities. The most common forms are *lupus, ulcers, gummata, lymphangitis;* the whole series of lesions to which dermatologists, after Darier, have given the name of *Tuberculides:* keloids, papulo-necrotic tuberculides of Barthélemy and de Brocq, verrucuous tuberculosis of Riehl and Paltauff, sarcoids of Boeck, psoriasis too, almost certainly (Sabouraud),[1] certain mutilating chill-blains of the ears in the aged, the angiokeratomas of Mibelli, erythema nodosum and purpura of adolescents.

A. LUPUS

Lupus is characterized, in the beginning, by one or more flat or prominent nodules which, by their confluence form more or less extensive lesions. The latter may or may not be elevated above the skin surface and may or may not be ulcerated. They are observed most commonly on the face, the parts of the body uncovered by clothing and on mucous membranes (*see Plate XI*).

That the nature of the nodules of lupus and of tuberculosis is the same was affirmed by Friedlander,[2] but we owe its demonstration

[1] Presse méd., 1917, i, 9.

[2] Virchow's Arch., 1874, **60**, 15.

particularly to the work of Hipp. Martin, de Leloir and Vidal,[3] Cornil, R. Pfeiffer, Doutrelepont,[4] and Unna.[5]

The lupus nodule develops histologically in the lymphatic spaces of the small nerve ganglia of the dermal papillae. It spreads along the sheaths of the small vessels which supply these ganglia, gradually involving the sebaceous and sweat glands which are smothered in a cellular mass. In this manner it extends its destructive action to the intercellular spaces which it dilates, to the connective and elastic tissue fibres and to the vessels and nerves of the whole area wherein it is diffused.

In the earliest stages of lupus lesions, giant cells are usually to be found well developed, large in size and with a crown of many nuclei surrounding a mass of clear protoplasm in which bacilli are rather few, often even very rare and degenerated. In the exudate from lupus ulcerations eosinophile cells are to be found.

The varied appearance presented by lupus depends in large part on the degree of alteration undergone by the nervous ganglia, and the nerves involved are more or less destroyed by the development of its mass. Factors of similar great importance are the depth to which the lupus mass reaches into the dermic tissue, the functional importance of the nerve or vascular branches implicated, the site of the lesion, the degree of virulence of the causal bacilli, and, finally and above all, the resistance of the individual.

Suppuration of lupus lesions is favored by the organisms of secondary infection (staphylococcus or streptococcus) which multiply in the lymph exudates. Tubercle bacilli stainable by Ziehl are extremely rare. At times not more than one is to be found in as many as ten sections. On the other hand, the acidophile and gram-positive granules stainable by Much's method are more abundant. It would seem therefore that, in the tuberculous nodules of the skin, the tubercle bacillus does not find a very favorable medium for its multiplication and that it there fairly rapidly undergoes alterations in form and virulence.

Jadassohn[6] published a very interesting observation on this question, which may be regarded as equivalent to a laboratory experi-

[3] Compt. rend. Soc. de biol., 1882, **34**, 700.
[4] Deutsch. med. Wchnschr., 1892, **18**, 1033.
[5] Ibid., 1898, **24**, Vereins-Beil., 280.
[6] Virchow's Arch., 1890, **121**, 210.

PLATE XI

1. Tuberculo-gummatous lupus of the nose and lips (from the model of a specimen in the Museum of the Hôpital St. Louis; service of Dr. Besnier).

2. Cutaneous tuberculosis. Tuberculous folliculitis of the back of the hand (Hôpital St. Louis; services of Drs. Hallopeau and Jeanselme). Autochrome photograph of a model.

3. Papillomatous cutaneous tuberculosis of the hand (Hôpital St. Louis; service of Dr. Lailler). Autochrome photograph of a model.

4. Scrofulo-tuberculous dactylitis, fungous synovitis of the right index finger (Hôpital St. Louis; service of Dr. Le Dentu). Autochrome photograph of a model.

I

II

III

IV

ment; it has to do with a woman contracting lupus at a point tattooed by her lover who shortly afterward died of phthisis. The lover had made use of his own saliva in the process of tattooing.

Although it is true that the inoculation into a guinea pig of a sufficiently large and properly ground-up fragment of lupus tissue gives rise always to the development of tuberculous lesions, the latter are in general benign; they progress slowly over a long period, manifest themselves only by a local adenitis near the point of inoculation, and the infection becomes generalized only after several months or even more than a year. This very evident attenuation of the bacillus cultivated in the dermic tissue explains why *no one has ever been able to inoculate lupus from one human being to another.*

The pathogenesis of the different varieties of lupus (*lupus planus, eccentric, nummular* or *discoid, maculosus; lupus exedens* or *ulcerative, tuberculo-gummatous, vegetant, lupus pernio* or *erythematosus*) is still none too well understood. It should be cleared up by experimental work. Such experiments are however difficult to carry out, since they can be properly done only with anthropoid apes whose dermic tissue is similar to that of man. The difficulty is still further increased by the fact that in the monkey the smallest inoculation of tuberculous virus leads promptly to generalized infection. Now it seems that lupus can appear and develop only in already tuberculous individuals who have latent or occult lesions, and it must be looked upon as a lesion of *reinfection.* The first requirement, therefore, in order to properly carry out an experimental study, would be the preparation beforehand of a certain number of apes, so that they might be rendered suitably resistant to tuberculous infection through preliminary inoculations of very attenuated but still viable bacilli.

We have more definite knowledge as to the relationship between lupus lesions and pulmonary tuberculosis in man. All clinicians realize the rarity of skin localizations where pulmonary or visceral tuberculosis is already present. *It is very exceptional to see a phthisical case become one of a lupus.* On the other hand, lupus cases frequently become tuberculous, *and phthisis is almost always the normal finale of the long calvary suffered by the victim of lupus.*

If glandulo-pulmonary tuberculosis seems to protect against lupus, the situation is otherwise as regards adenitis and the other so-called scrofulous manifestations which are generally the outcome of infections by attenuated bacilli. These latter precede, or accompany the

evolution of the lupus, or are often, through the orifices of their fistulae, its point of departure.

Lupus may appear at any age, but is exceptional before the third or fourth year, and rarely begins after the thirtieth year. The greatest frequency is found between the ages of six and ten years, according to E. Vidal,[7] Brocq, Kaposi and the majority of dermatologists.

Localizations upon the face, particularly on the alae nasi, are by far the most commonly observed (in about 65 per cent of cases according to Raudnitz);[8] this is due perhaps to the facility with which the bacillus may be inoculated into the skin in the process of shaving, kissing, scratching small acne pustules, etc.

After the face, the neck, the hands and the feet are most often affected. In certain occupations which expose the individuals more particularly to dermatoses, attention has been called to the great frequency of sclerous papillomatous lupus of the hands (*see Plate XI*). Rothe and Bierotte[9] studied 28 cases of lupus with a view to determining whether the bacilli were of *human* or *bovine* origin. A piece of skin was taken from each subject and inoculated into guinea pigs. The type of bacillus recovered from the organs of the animals was later determined by culture and by inoculation into the rabbit. Thus they found the *human type in 82 per cent* of cases and *the bovine type in 14.3 per cent.* It may be said therefore that the majority of cases of lupus are caused by the human tubercle bacillus.

Analogous experiments have been performed by other investigators. They will be found described further in the book (*Chapter XXV*).

B. TUBERCULOUS ULCERS

These lesions are characterized by superficial losses of substance. They may gradually extend, but usually they confine themselves to the vicinity of their inception. Almost always they are a secondary manifestation in individuals who have old pulmonary or intestinal lesions. But occasionally they are observed as primary lesions, for example in children ritually circumcised. The ulcers are then accompanied by a characteristic engorgement of the corresponding gland group, which is exceptional in non-ulcerative lupus cases.

[7] *Du lupus*, Paris, 1879, Delahaye & Cie.

[8] Veröffentl. d. R. Koch Stift. z. Bekämpf. d. Tuberk., 1913, H. 7/8.

[9] Ibid.

Most commonly *the tuberculous ulcer* is the outcome of a *local reinfection* of the skin by infectious saliva or by fecal matter containing bacilli. Thus it often appears in the anal region or upon the lips, especially the lower lip which is more exposed to the mouth secretions.

When the tuberculous ulcer is *secondary*,—as is usually the case in individuals with a long standing infection, or in those rendered more resistant by a latent glandular infection,—it loses its tendency to sloughing and rapid spreading and assumes the characteristics of a verrucous or vegetating lesion, without engorgement of the neighboring lymph nodes.

The histology of secondary tuberculous ulcerations was studied by Vallas in Renaut's laboratory in 1887. According to him, the lesions begin in the dermis, and not in the glands, in the form of granulations made up of epithelioid cells with or without giant cell formation. In the center of the inflammatory tissue, a large number of bacilli are found arranged in rows, particularly at the site of the hypertrophied papillae at the margin of the ulcer. Round about the early tubercles are often found elastic tissue fibres broken up into a large number of granules and bundles of connective tissue in a state of gelatinous degeneration.

Bacilli are often lacking in the exudate from the ulcer surface, but the granulations in the wound contain many of them. These bacilli are usually attenuated in virulence.

C. TUBERCULOUS GUMMATA

Under this heading are designated the nodular formations of the skin whch develop either in the chorion, or between the chorion and the superficial aponeurosis, or deep in the subcutaneous cellular tissue, and from which there oozes a bacillus-containing purulent exudate. When these nodules are small, they have exactly the structure of '*tubercules crus*' of the lungs, in that they are formed by a coalescence of several granulations or miliary tubercles. Later they increase in size, become soft and bleed at the slightest touch. They show, under the microscope, a peripheral zone of newly formed cells disseminated in the interstices of the connective tissue, and also obliterated blood vessels and giant cells.

The walls of the gummata are their vital part. Through them they increase in size and extent, while the centers undergo caseous soft-

ening and contain a serous grumous pus, of the color of café au lait, and poor in bacilli. In contrast to the gummata of syphilis, they show no tendency to fibrous transformation.

Gummata of the dermis are observed, at times isolated, again collected together sheet-wise or with finger-like projections, upon any part of the surface of the body whatsoever, but they are more common on the sides of the face, especially along the ascending ramus of the lower border of the inferior maxilla, on the thorax or on the limbs. They occur at all ages, but most commonly in adolescents who already have other tuberculous lesions. They start occasionally from an axillary cold abscess having no connection with the sweat glands, but of lymph node origin, or from a tuberculous lymphangitis.

D. TUBERCULOUS LYMPHANGITIS

Tuberculous infection of the skin, resulting either from a small superficial wound acquired for instance in the course of an autopsy, or from the contamination of tattoo prickings by bacillus-containing saliva, leads to the formation of hard nodules of yellowish white color, which obstruct the lymphatic canals and develop in their endothelium. The nodules soon soften at the centre. Their rupture permits the escape of serous pus containing a very few bacilli, which are in general of low virulence.

This lymphangitis takes the form of a string of beads (*en cordon*), or occurs in isolated foci. Now and then, but rarely, it develops into lymphangiectatic elevations or linear swellings (*bourrelets*) (Hallopeau and Goupil),[10] with false fluctuation, and of a fungous or soft consistency.

E. TUBERCULIDES

Under this heading, Darier[11] includes a whole group of dermatoses comprising *lichen scrofulosorum*, the *folliclis* and *acnitis* of Barthélemy or *papulo-necrotic tuberculides*, *acne cachecticorum* of Hebra and Kaposi, *acne scrofulosorum* of Fox, certain *agminated papulo-pustular lesions* described by Thibierge and by Hallopeau, the different varieties of *lupus erthematosus*, *erythema induratum* of Bazin, *angiokeratoma* of Mibelli, *pityriasia rubra* of Hebra, *eczema scrofulosorum* of Boeck, and certain forms of disseminated *lupus* and of lupus *in multiple plaques*.

[10] Société de dermatologie, July 10, 1892.
[11] Leçons cliniques de l'Hôpital Broca, 1905–1907.

These lesions are found almost exclusively in subjects already affected with other varieties of tuberculosis, especially the glandular or indolent forms. They present histologically the appearance of tuberculous nodules, but often show neither giant cells nor stainable bacilli, and their inoculation, with rare exceptions, gives negative results. This is probably due to the fact that the few more or less altered bacterial elements which they may contain have lost all virulence.

The most curious form of tuberculid is *lichen scrofulosorum*, whose site of election is the trunk (back, chest or abdomen), less often the arms or legs. It presents itself in the form of an eruption of rounded papules, in size from the head of a pin to that of a millet seed, isolated or covering large areas, in concentric circles now and then, rough and granular to the touch. Each papule, according to Hebra, is situated at the orifice of a hair follicle and is composed of a thick mass of epidermis which can be detached without causing any bleeding. The histological structure of the papules is that of the miliary tubercle. At times bacilli are found in them and, in all cases, the individuals affected react frankly to tuberculin. This characteristic, furthermore, is one which is common to all true tuberculides and, naturally, to all forms of tuberculosis of the skin.

Jadassohn,[12] Ehrmann[13] and the majority of dermatologists are inclined to think that lichen scrofulosorum is of hematogenous origin. B. Lipchutz[14] concluded from his experiments that it is almost always due to bovine bacilli, in contrast to lupus.

Many investigators have tried to produce experimental cutaneous lesions presenting the essential characteristics of the tuberculides. Kraus and Grosz,[15] Baermann and Halberstadter[16] attempted this in 1905 in the monkey, but they succeeded only in giving generalized tuberculosis to their animals with local lesions of lymphatic infiltration and verrucous ulceration.

Gougerot and Laroche[17] had better results with a very simple technique:

The back of a guinea pig was epilated over an area of 2 to 3 cm. by

[12] Arch. f. Dermat. u. Syphilis, 1914, **119**, 10.
[13] Ibid., 1914, **119**, 83.
[14] Ibid., 1914, **120**, 387.
[15] Wien. klin. Wchnschr., 1907, **20**, 795.
[16] Berl. klin. Wchnschr., 1906, **43**, 199.
[17] Arch. de méd. expér., 1908, **20**, 581; **21**, 324.

pulling out the hair with the fingers. A small mass of pure culture from potato medium was deposited on the epidermis and smeared over it with pressure and friction, a heavy platinum wire or glass rod being used.

By using different strains of tubercle bacilli, and particularly the one maintained at the Pasteur Institute under the name of "*Bovine lait, de Nocard*," Gougerot and Laroche claim to have obtained tuberculides which occasionally had the appearance of lichen scrofulosorum and more often that of human papulo-necrotic tuberculides.

In a guinea pig rendered tuberculous and treated with tuberculin as a preliminary measure the same investigators have seen a keloid, 25 mm. long, 5 mm. wide and histologically identical with human keloids, develop slowly at the very point where the tuberculin had been injected. In one of its segments, the deep portion showed scattered tuberculous nodules, as in the keloid margins of certain cases of lupus.

The interesting experiments of Gougerot and Laroche appear open to question as to the interpretation of the results. It seems however that one should agree with them in regarding the majority of tuberculides as lesions *without tubercles* (*non-folliculaires*), produced—in the skin of individuals already harboring latent or occult glandular tuberculosis,—by the cutaneous elimination of bacilli or the toxic products elaborated by the tubercle bacillus.

Et. Burnet[18] succeeded in isolating from an old sluggish cutaneous lesion, a bacillus of very low virulence for the guinea pig and monkey. From certain intradermic inoculation experiments which he made with this bacillus, in collaboration with Ch. Mantoux, he is inclined to think that the skin perhaps, of all the body tissues, lends itself best to a *spontaneous attenuation* of the tuberculous virus. "It is not a matter of indifference to a tuberculous individual," says he, "that the skin has been affected before the lung or the lung before the skin, and his pulmonary tuberculosis is probably not the same as it would have been were the skin not involved."

In studying the problem of immunity to tuberculosis, we shall have occasion to return to this subject (*Chapter XLII*).

[18] Ann. de l'Inst. Pasteur, 1912, **26**, 868;—Bull. Soc. d'étude scient. de la tuberc. 1913,, June.;—Compt. rend. Soc. de biol., 1912, **73**, 384.

CHAPTER XVIII

TUBERCULOUS BACILLEMIA

The protean character of tuberculous infection, and the many clinical and experimental facts tending to prove that the virus usually penetrates into the body by way of the lymphatic channels, indicate that the blood circulation plays an important rôle in the dissemination as well as in the localization of the bacilli. One would therefore expect to find the organisms in the blood, at least at the beginning to the infection, and at times even during its course. Such in fact is the case.

As early as 1866, Villemin demonstrated that blood, taken from the femoral artery of a tuberculous rabbit or from phthisical patients by means of cupping, and inoculated subcutaneously into normal rabbits, transmitted the tuberculous infection. W. Marcet successfully repeated the same experiment in 1867. And Weichselbaum[1] later (1884) succeeded in demonstrating the bacilli by microscopic examination of the blood in 3 cases dying of acute miliary tuberculosis. Meister, Meisels,[2] then Lustig, Rutimeyer, Gosselin, and Vaquez, found them in similar cases, either after death or antemortem. Other observers had the same results (Sticker, Jeannel,[3] Galtier, Gaertner,[4] Bergkammer, Ulcacis, Doutrelepont, Ettlinger,[5] and others). But, despite the efforts of Landouzy, who as early as 1882 had made acute tubercle bacillus infection a clinical entity under the name of *typhobacillose*, it was not admitted that, aside from miliary tuberculosis and a few rare cases of chronic phthisis (about 2 per cent), the blood could contain bacilli.

Numerous recent studies show that this point of view was justified only by the then imperfect methods of examination.

The method of inoculation used in experimentation was first to directly inject rabbits or guinea pigs intraperitoneally or subcutane-

[1] Wien. med. Wchnschr., 1884, **34**, 333; 355.
[2] Ibid., 1884, **34**, 1149; 1187.
[3] Internat. Congr. on Tuberc., Paris, 1888.
[4] Ztschr. f. Hyg., 1893, **13**, 101.
[5] Thèse, Paris, 1893.

ously with defibrinated blood of animals of the same species previously tubercularized hypodermically or intravenously. Some, like Jeannel and Gaertner, inoculated thus 10 to 40 gms. of blood, or even, as did Kuss, half of the blood mass of the animal (guinea pig); others, like Nocard,[6] used only from a quarter of a cubic centimeter to 5 or 10 cc. of blood from the guinea pig or tuberculous patient. The results obtained were extremely variable and in general led to the conclusion that bacilli introduced into the veins of the rabbit become rapidly fixed in the tissues and soon disappear from the circulation (after 4 hours according to Nocard, in from 1 to 6 days in Gaertner's opinion). It also appeared that blood taken at autopsy or during life from phthisical or miliary cases is frequently infectious for the guinea pig, even in small doses (from 1 to 11 cc.).

F. Bezançon, Griffon and Philibert[7] in 1903 applied their concentration method to the direct microscopic search for bacilli in the blood. They succeeded on two different occasions in finding bacilli in a case of pulmonary tuberculosis with hemoptysis. Their technique was as follows:

To 5 cc. of blood in a mortar (clot and serum) 5 cc. of distilled water and 5 drops of normal sodium hydroxide solution were added. The clot was ground until dissolved in the liquid, 2 cc. of distilled water were added, and the whole boiled in a porcelain dish for 10 minutes. Then, after centrifuging, the sediment was spread upon slides and stained by Ziehl.

André Jousset, in a communication before the Société médicale des hôpitaux (January 9, 1903), was the first to propose taking the whole or a large part of the blood of a tuberculous animal, or of a coagulable sero-fibrinous exudate (from pleurisy for example), and to digest it artificially in order to centrifuge and collect the bacterial elements which might be contained therein.

This procedure, to which its author gave the name of *inoscopy* (*inoscopie*), consists in digesting the blood or sero-fibrinous clot by a sort of gastric juice whose composition is here given:

	gms.
Pepsin scales (Titre 50 of the French Pharmacopocia)	2
Sodium fluoride	3
Pure glycerin	10
Hydrochloric acid (22° Beaumé)	10
Distilled water	1 liter

[6] Bull. Soc. centr. de méd. vét., 1885.

[7] Compt. rend. Soc. de biol., 1903, **55**, 35; 203.

The blood must be collected aseptically. After coagulation it is washed with sterile distilled water in order to hemolyze the majority of the red cells. The clot is allowed to fall into a large mouth flask and an equal volume of the digestive fluid is added. The whole is then left in an incubator at 38°C. for 2 or 3 hours, or on a water bath at 50° for a half hour. With shaking from time to time, dissolution is soon almost complete. The mixture is then centrifuged and the supernatant fluid discarded. The sediment is spread upon slides, dried and stained with Ziehl. The tubercle bacilli are seen in the cellular debris.

This method of Jousset, with which he obtained 11 positive results in 35 examinations of blood from cases of pulmonary tuberculosis, that is, in 31 per cent, aroused much criticism. André Bergeron,[8] Bezançon, Gouget, then Hugo Pribram showed that it is subject to many errors, the chief of them being that it does not permit the differentiation of tubercle bacilli from the acid-fast microörganisms which are frequently found in the blood of healthy individuals, and which have their origin in the intestine or on the skin surface.

The same criticism might be made of another method devised by Lesieur,[9] which consists in applying to the carefully cleaned skin of the patient 3 or 4 fat leeches previously washed in sterile water, in allowing the leeches to gorge themselves and then decapitating them to collect the blood in a centrifuge tube (*procédé de la sangsue*).

Loeper and Louste,[10] Nattan-Larrier and Bergeron[11] made use of a more practical and more certain method, based on the principle of hemolysis.

Loeper and Louste aspirate one volume of blood from a vein directly into a 20 cc. syringe containing 2 volumes of 33 per cent alcohol. The mixture is immediately put into a suitable tube and centrifuged, and the sediment then stained and examined.

Nattan-Larrier and Bergeron likewise aspirate the blood from a vein or from an animal's heart and mix it immediately in a sterile flask with 20 volumes of distilled water. In this way they obtain a clear fluid free from fibrin clots, which is divided in tubes and centrifuged at once for 10 to 15 minutes. The sediment, of which there is a very little under these circumstances, is spread upon slides and stained by the usual technique after drying with heat.

[8] Thèse, Paris, 1904.

[9] J. de physiol. et de path. gén., 1904, **6**, 875.

[10] Arch. de méd. expér., 1905, **17**, 301.

[11] Presse méd., 1905, i. 371.

Lafforgue[12] also aspirates from the heart of a tuberculous guinea pig 1 cc. of blood which is immediately mixed with 20 drops of 2 per cent sodium citrate solution and centrifuged. The sediment may be inoculated into normal guinea pigs. He thus obtained 2 positive results in 4 trials.

André Jousset,[13] by altering his original inoscopy technique, demonstrated that the fluid blood may very advantageously be mixed with 20 to 25 times its volume of alcohol-HCl solution made up as follows:

	cc.
Alcohol (25 per cent)	500
HCl (22° Beaumé)	1

Blood cells and the protoplasm of the leucocytes are perfectly dissolved without the formation of an albuminous precipitate and without any alterations in the bacilli. The latter are easily recovered among the leucocytic nuclei after centrifuging the mixture and staining and counterstaining the sediment.

With this technique inoculation controls may be made. A. Jousset used it to study a special form of guinea pig septicemia which he observed following subcutaneous injection of certain strains of human tubercle bacilli.

Léon Bernard, Debré and Baron[14] collect 10 cc. of blood in a sterilized tube containing 20 cc. of 30 per cent alcohol and, in order to hasten the laking of the red cells, they add about 30 cc. of 40 per cent alcohol. After shaking for a moment the mixture is centrifuged for a half hour. The sediment is then emulsified in 40 cc. of 40 per cent alcohol, to which have been added one or two drops of a 10 per cent alcoholic solution of sodium hydroxide, and centrifuged a second time. There is then formed a scanty sediment which is spread upon 2 or 3 slides and stained by Ziehl.

R. C. Rosenberger,[15] and Forsyth[16] prefer to dispense with centrifuging. Ten to 30 cc. of blood (or more if possible), collected aseptically in physiological salt solution containing 2 per cent of sodium citrate, are left for 24 hours in a refrigerator. The sediment falls

[12] Compt. rend. Soc. de biol., 1909, **67**, 96.
[13] Compt. rend. Acad. des sci., 1908, **146**, 1060.
[14] Bull. Soc. d'étude scient. sur la tuberc., 1912, Nov.
[15] Am. J. Med. Sci., 1909, **137**, 267; New York Med. J., 1909, **89**, 1250.
[16] Brit. Med. J., 1909, i, 1001.

spontaneously under these conditions. It is drawn up in a pipette and spread thickly on slides which are dried in an incubator and then dipped into sterile distilled water in order to hemolyze the red cells. The preparation becomes clear and, after slow drying in order to avoid the formation of coagulum, it is gently fixed over a Bunsen burner and stained cold by the method of Ziehl.

Rosenberg applied this method to 312 patients and would have us believe that he found bacilli in all cases of tuberculosis at different stages, even in glandular and bone forms. Moreover he considers that a bacillemia is present in every case of tuberculosis. He even believes that the tubercle bacillus exists now and then in the blood of subjects in whom clinical examination discloses no apparent lesion. He found it in 6 of 112 cases in whom tuberculosis could neither be diagnosed nor suspected.

In Germany several workers strongly recommend *antiformin*, for the use of which they have a variety of methods, the principal ones being those described by Sturm,[17] Staubli, G. Liebermeister,[18] Schnitter, Kurashige, Zeissler and Rumpf. Speaking generally they consist in treating 5 to 30 cc. of blood with 5 times its volume of a 0.2 per cent aqueous solution of neutral potassium oxalate, or with 2 volumes of 3 per cent acetic acid, or with one volume of a 3 per cent citric acid solution. The blood is shaken and is at once laked. After centrifuging, the supernatant fluid is poured off and the sediment taken up in 1 cc. of distilled water so as to thoroughly break up any small clumps. Pure antiformin is then added drop by drop (1 to 3 drops are sufficient), or 3 volumes of 15 per cent antiformin,[19] with stirring until clearing is complete. The tube is then filled with 60 per cent alcohol and centrifuged. Part of the sediment is used for slide preparations; the remainder is diluted with a little physiological salt solution and injected into the peritoneum or under the skin of guinea pigs.

All glassware used in these manipulations should have been kept for 24 hours in fuming sulphuric acid, then washed in a solution of

[17] Beitr. z. klin. der Tuberk., 1911, **21**, 190; 239.

[18] Med. Klin., 1912, **8**, 1018.

[19] Several hours in antiformin (as much as 24 hours according to some authors) do not alter the form of the bacilli; but to avoid modifying their viability where inoculations are to be made, it is wise not to exceed a couple of hours.

boiling soda and rinsed with sterile distilled water, since tap waters frequently enough contain acid-fast bacilli (Beitzke,[20] Schern and Dold[21]). It is essential moreover that the skin of the subject or animal from whom the blood is taken should be well disinfected with alcohol or ether. Thus are avoided the accidental contaminations by acid-fast microörganisms which are always numerous upon the skin and in dust.

With this technique, more or less modified according to the individual author, it is found that *tubercle bacillus bacillemia is frequent in all forms of tuberculosis.* Schnitter[22] says that it is present in 31.6 per cent of cases of tuberculosis.

Liebermeister,[23] in second stage tuberculosis, demonstrated the bacillus in 30 per cent of cases; Lippmann in 33 per cent of all his cases, and in the third stage in 53 per cent. Manouen recovered the bacilli 12 times in 15 patients and even found them in some incipient cases. Susuki and Takaki[24] have 478 positive cases in 516. They claim that there is an absolute parallelism between the skin reaction and the presence of bacilli in the blood.

Kurashige[25] goes further; his experiments showed the bacillus constantly present in the circulating blood in 155 phthisical cases at different stages. In the investigation of another series of 20 cases of phthisis, he examined the blood on 1 to 8 occasions during the course of 12 weeks. Among 114 tests so made, the bacillus was found in 104 instances. In his opinion, these results lead to the conclusion that the blood of phthisical cases almost always contains bacilli, even in the moderately severe or mild cases.

Rumpf,[26] too, with the aid of Zeissler, found bacilli in 100 per cent of his 25 patients. Erich Rosenberg[27] examined the blood of 19 pulmonary cases with only 1 negative result. In 3 other suspected patients, the same author twice found the tubercle bacillus in the blood, and these two individuals soon afterward showed definite pulmonary tuberculosis, while the third, who was later seen on

[20] Berl. klin. Wchnschr., 1910, **47**, 1451.
[21] Arb. a. d. k. Gsndhtsamte, 1912, **38**, 205.
[22] Deutsch. med. Wchnschr., 1909, **35**, 1566.
[23] Med. Klin., 1912, **8**, 1018.
[24] Centralbl. f. Bakt., 1912, **61**, 149.
[25] Ztschr. f. Tuberk., 1911, **17**, 347; 1911/12, **18**, 430; 433.
[26] München. med. Wchnschr., 1912, **59**, 1951.
[27] Ibid., 1913, **60**, 404.

several occasions, remained free from disease. In other subjects without previous history, without clinical signs of infection and in whom radioscopic examination was negative, examination of the blood failed to show the bacillus.

Moreover, Klara Kennerknecht[28] found bacilli 68 times in the blood of 68 children clinically tuberculous, 18 times in 20 doubtful children and 23 times in 31 others apparently healthy. Only 12 of the latter gave a positive cutaneous reaction with tuberculin. Microscopic examination was confirmed in 13 cases by inoculation of guinea pigs.

Before the Society of Medicine of Berlin, F. Klemperer[29] reported that B. Fischer (of Frankfort), inoculating guinea pigs with lymph from the thoracic duct of subjects dying of intestinal tuberculosis, found bacilli in three-quarters of the cases. He himself, in 16 cases of pulmonary tuberculosis, was able in 11 to demonstrate the bacillus in the blood by experimental inoculation.

Finally Ritter, Sturm,[30] Krause-Hannover,[31] Hilgermann and Lossen[32] found the bacilli in the circulating blood in 20 to 50 per cent of cases which they studied. Inoculation into the guinea pig was positive, even in certain cases where the microscope revealed nothing. The bacillus was never found in the blood of healthy individuals.

Brandes and C. Man had the same results in patients with surgical tuberculosis.

All these facts are of considerable importance, since they indicate that *tuberculous infection is a bacillemia from the outset,* and prior to any local lesion, the development of the latter being more or less delayed according to the intensity of the infection, the virulence of the bacilli and the resistance of the individual.

It is only fair to recognize that certain other investigators have not had such impressive results. H. Lüdke,[33] for instance, succeeded only 3 times in 14 in infecting guinea pigs by injecting them intravenously with 4 to 7 cc. of whole blood taken by venous puncture from chronic pulmonary cases. Fraenken, in 51 cases of phthisis (32 in the third

[28] Beitr. z. klin. der Tuberk., 1912, **23**, 265.

[29] Ibid., 1914, **31**, 76;—Ztschr. f. klin. Med., 1914, **80**, 82.

[30] Ibid., 1911, **21**, 239.

[31] Ztschr. f. Tuberk., 1911, **17**, 436.

[32] Deutsch. med. Wchnschr., 1912, **38**, 895.

[33] Wien. klin. Wchnschr., 1906, **19**, 949.

stage and 19 in the second), had only 7 positive results (of which 5 were in the third stage) in injecting 10 cc. of blood. Hilgermann and Lossen, using the same technique as Kurashige, found only 17 positives among 64 cases in various stages. F. Klopstock and Erich Seligmann[34] had nothing but negative results among 49 phthisical patients in all stages.

In the opinion of Lydia Rabinowitsch,[35] bacillemia occurs in 30 per cent of tuberculous cases, even in the early stages of the infection. P. Ranström[36] thinks it is present in 25 per cent of cases; the appearance of the bacilli in the blood stream, according to him, being coincident with elevations of temperature.

On the other hand Hans Kohn, J. Elsaesser[37] in Germany, Sabrazes, Eckenstein and Muratet,[38] Léon Bernard, R. Debré and Baron[39] in France obtained much lower percentages; Elsaesser, 3 cases in 41, Léon Bernard and his collaborators 4 cases in 41. Of the latter 4 positive cases, 1 was of meningitis complicating chronic tuberculosis, 2 were miliary cases and 1 was of pulmonary phthisis with cavity formation. But it is important to note that the technique here employed for collecting the blood is open to criticism. It was taken in too small quantity (12 to 15 cc. only), in paraffined tubes to avoid coagulation; after centrifuging, all of the plasma was inoculated subcutaneously into one guinea pig, while the cells were inoculated separately under the skin of a second.

In the United States, Jane L. Berry,[40] in 51 cases examined, had uniformly negative results.

Rist, Armand Delille and Lévy Bruhl[41] likewise tried to find bacillemia in 50 tuberculous cases and had positive results only 3 times by direct examination; in only 2 of these cases could the finding be confirmed by guinea pig inoculation. But here again objection can be raised, since the control animals were inoculated with only 6 to 7 cc. of whole blood, a quantity both insufficient and dangerous,

[34] Ztschr. f. Hyg., 1913, **76**, 77.
[35] Berl. klin. Wchnschr., 1913, **50**, 110.
[36] Deutsch. med. Wchnschr., 1912, **38**, 1535.
[37] Beitr. z. klin. der Tuberk., 1913, **26**, 367.
[38] Compt. rend. Soc. de biol., 1909, **66**, 803.
[39] Bull. Soc. d'étude scient. tuberc., 1912, Nov.
[40] J. Infect. Dis., 1914, **14**, 162.
[41] Bull. Soc. d'étude scient. tuberc., 1913, April.

insufficient because the number of bacilli contained in so small a portion of the blood must be extremely small or nil, at any rate so reduced that it is incapable of transmitting active tuberculosis to a guinea pig in the course of a few weeks; dangerous because unheated human blood or serum is of itself toxic for the guinea pig in a quantity of about 8 cc. The same criticism might be made of the earlier researches of P. Nobécourt and Darré on tubercle bacillus bacillemia in children. Only 4 times in 40 cases were they able to obtain positive results by intraperitoneal injection into the guinea pig of 3 cc., 3.5 cc., 5, 6 and 7 cc. of blood withdrawn from the elbow vein or collected by means of leeches. The 4 positive cases were of acute tuberculosis, one of them in a girl of 14 years who, after having had a latent tracheo-bronchial adenopathy, developed a bacillemia with multiple localizations including arthropathies, endocarditis and pleuro-pulmonary congestion, from all of which she recovered completely.

Observations of cases of this sort, going on to cure despite the demonstration of tubercle bacilli in the blood, are now no longer rare. E. Ausset and M. Breton[42] have collected several. They will probably increase in number when the bacilli are sought for with a more satisfactory technique and clinicians are better informed as to the symptoms and signs by which the bacillemia may manifest itself.

By means of an ingenious technique making possible the transfusion of blood from a tuberculous guinea pig to a healthy one, L. Massol and M. Breton,[43] at the Pasteur Institute at Lille, were able to definitely determine the frequency, duration and intensity of blood infection following the different modes of inoculation.

After having injected equal doses of virulent bovine bacilli (1 mgm.) into two series of 10 guinea pigs of the same weight, in the one set into the jugular vein, in the other under the skin of the thigh, they transfused the blood of the first series of animals to healthy guinea pigs at varying intervals—30 minutes, 1, 2, 4, 6, 18, 42, 90 hours, 11 and 15 days after infection; the blood of the second series after 1, 2, 4, 10, 15, 30, 40 and 60 days. The control pigs, inoculated intravenously, died as a rule in 20 days; those inoculated subcutaneously, in 60 to 70 days. Each normal guinea pig transfused received about 10 cc. of blood from the tuberculous animal donor (approximately one-quarter of the whole volume of circulating blood).

[42] Compt. rend. Soc. de biol., 1914, **76**, 70.

[43] Ibid., 1913, **74**, 21; 792; **75**, 455.

Carrying out the experiments under these conditions, it was found that all of the guinea pigs which had received, either the blood from the animals of the first series infected intravenously, or that from the animals of the second series, died with a generalized tuberculous infection after 30 to 100 days (*fig. 12*).

In other experiments, the same workers saw that after subcutaneous inoculation of only $\frac{1}{10}$ of a milligram of virulent culture, the blood of guinea pigs is *always infectious* for transfused normal guinea pigs when the transfusion is performed from the 1st to the 47th

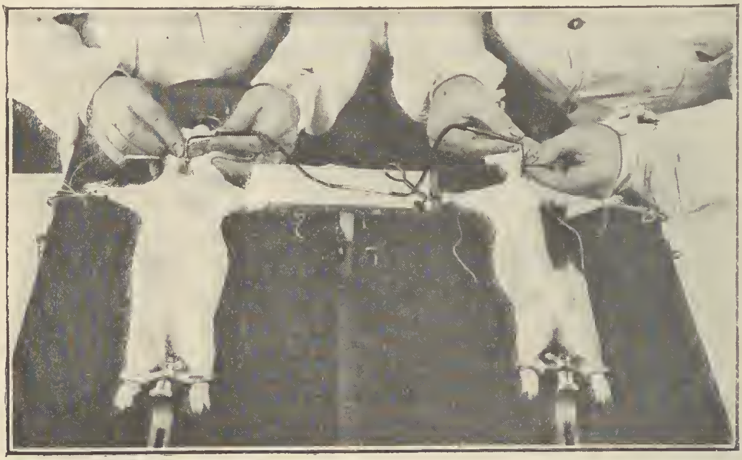


Fig. 12. Transfusion of Blood from the Carotid of a Tuberculous Guinea Pig to the Jugular Vein of a Normal Guinea Pig, in Order to Study Tuberculous Bacillemia at Different Stages of Infection

day after the original infection, and that, in the recipient animals, the lesions are the more discrete the fewer the bacilli transported by the blood.

If infection of the donor has been accomplished with an even smaller dose, $\frac{1}{100}$ to $\frac{1}{100,000}$ of a milligram, bacillosis manifests itself in the recipient animals after 11, 30, 41 and 46 days. But the reactions are then limited to the lymphatic organs; only exceptionally do they involve the abdominal and thoracic viscera, and the bacillemia reaches a maximum intensity at about the 10th day.

André Jousset,[44] Léon Bernard, and Debré and Baron[45] have reported findings permitting similar conclusions.

In Germany, Neumann and Wittgenstein[46] succeeded in demonstrating bacilli in the blood of a dog 35 days after intravenous injection. Bongert, in the rabbit, recovered them between the third and twenty-fourth days, and Titze,[47] using 6 goats and 7 cattle, observed their persistence in the circulation and in the muscles up to the twenty-third day after the intravenous inoculation of 1 mgm. of a culture of bovine origin. In the bullock, Binder[48] proved that after intravenous inoculation a certain number of bacilli remain in the blood circulation from the third day to the eleventh day; that they then disappear, to reappear from the seventeenth to the thirty-first day. After subcutaneous inoculation, he found them on the twenty-fourth, fortieth and seventieth days.

The experimental method therefore proves that in a tuberculous infection, even though mild, the blood almost always contains bacilli, and if the observers who have sought for them in man disagree as to their constant presence, it is probably due to the fact, as Jousset remarked, that their inoculation experiments have been carried out with altogether too small a quantity of blood, and perhaps to the fact that a relatively considerable quantity of antibodies present in the blood is injected along with only a few bacilli. I have proved in fact with L. Massol that intravenously, more than 10 bacilli of average virulence must be inoculated into guinea pigs if the latter are to be rendered tuberculous. With a smaller number of bacteria, glandular lesions may now and then be produced,—although exceptionally,—but they remain benign and harmless for months, pass unnoticed at autopsy and leave the animals apparently free from infection.

Another proof of this frequency, although an indirect one, is furnished by the findings of different observers who have sought for bacilli in tissue or in the pulp of different organs, whether during life, or after the death of tuberculous subjects (Galtier, Chauveau and Arloing,[49] Schnitter, Peuch, Bang, André Jousset,[50] Léon Kind-

[44] J. de physiol. et de path. gén., 1904, **6**, 909.

[45] Bull. Soc. d'étude scient. tuberc., 1913, May.

[46] Wien. klin. Wchnschr., 1906, **19**, 858.

[47] Arb. a. d. k. Gsndhtsamte, 1913, **43**, 505; 520.

[48] Berl. tierarztl. Wchnschr., 1913, **29**, 513.

[49] Congr. Vétérinaire, 1885.

[50] Arch. de méd. expér., 1904, **16**, 521.

berg,[51] and others). For example, Landouzy and Loederich,[52] studying a nodule of erythema nodosum excised from a living patient, succeeded in transmitting tuberculosis to a guinea pig by grinding up and inoculating this nodule. For the first time, they brought experimental proof of the tuberculous nature of this very characteristic eruption, which is manifestly the result of a bacillemia.

P. Ameuille and Léon Kindberg[53] likewise, by systematically inoculating pieces of kidney, skin muscle, hypophysis, etc., apparently healthy and removed under the best conditions from tuberculous subjects soon after death, obtained a very high percentage of positive results (57 per cent), *when by histological examination it was impossible to find any bacilli.* It must of course be admitted that the infecting power of these organs can be due only to the presence of virulent elements borne by the blood stream during life.

All of these facts suggest that one must be very reserved in the estimation of negative results of inoculation experiments with very small quantities of blood from tuberculosis cases and that in reality in the tuberculous, even at the onset of the infection, bacillemia is very much more common than has been thought up to the present.

This bacillemia, however, does not in any way signify a great number of localizations, since the bacilli, contained within the leucocytes, circulate in fluids more or less rich in antibodies. Indeed, these leucocytes themselves are producers of antibodies and may successfully resist intoxication by the secretory products of the bacilli. They but serve in a sense to mechanically convey the organisms about the body, until such time as they are ultimately eliminated with the cellular waste products (pigments, etc.) by way of the bile ducts and intestine.

With this in mind it will be understood that generalized tuberculosis or multiple localizations may be produced only in cases of massive infection, derived either from an abundant and repeated absorption of virulent cells, or from the abrupt discharge, into the blood current, of the contents of a cheesy tubercle filled with bacilli. Except in such cases, which fortunately are infrequent, tuberculous bacillemia remains fairly limited and the processes of defence through the secre-

[51] Thèse, Paris, 1913.
[52] Bull. Acad. méd., 1913, **70**, 400.
[53] Bull. Soc. d'étude scient. tuberc., 1913, April.

tion of antibodies are sufficiently powerful to overcome the bacilli carried about in the blood circulation *in small number* and *intermittently.* These are then disposed of as are harmless foreign bodies which can be eliminated along the normal paths of excretion.

In the light of this interpretation, which is sustained by all our clinical and experimental observations, it is obvious that no definite conclusion as to prognosis of tuberculous infection in man or animals can be drawn from the fact that there exists a more or less intermittent and more or less intense bacillemia.

CHAPTER XIX

RÔLE OF HEREDITY IN TUBERCULOUS INFECTION

Transmission of the Bacillus by the Parents.—Hereditary Dystrophies.—Specific Predisposition and Family Contagion

Until Villemin had demonstrated that phthisis is produced by a virus both infectious and inoculable, it was only natural that the frequency with which the disease successively attacks different members of the same family, often at an early age, should have imposed the belief of its hereditary transmission. The preponderating influence of heredity in the etiology of tuberculosis was accepted as dogma to the extent that Laennec himself, despite his keenness of observation, scarcely dared express a doubt. In his *Traité de l'auscultation médiate* he wrote, "A too common experience proves to all physicians that children of phthisical parents are more frequently attacked by this disease than are others. However, in this respect there are fortunately many exceptions; one fairly often sees families in which only one or two children in each generation become phthisical. On the other hand large families of children are occasionally observed to be destroyed by pulmonary phthisis, where the parents themselves have never been affected by the disease. I knew such a one in which both the father and mother lived more than 80 years and died from acute disease, after having lost 14 children, between the ages of 15 and 35 years, by pulmonary phthisis; all of them very robust children whose general condition would indicate no tendency to the disease. The fifteenth, born thin and delicate, showing all the constitutional signs usually associated with predisposition to pulmonary phthisis, went through several severe attacks of hemoptysis and on sundry occasions appeared infected with phthisis. Nevertheless he is the only survivor and is today 48 years old."

And even nowadays, many physicians succeed only with difficulty in emancipating themselves from prejudice in favor of the heredity of tuberculosis, while the belief is so wide-spread among the general

public that it will probably require long efforts of education to replace it with scientific truth.

Following the fundamental discoveries of Villemin and R. Koch, careful clinical observation and animal experimentation established the circumstances in which infection by the tubercle bacillus or its toxic products may affect the offspring of tuberculous individuals.

Direct transmission of the bacillus through the parents may take place:

1. In the ovum:

(*a*) Before impregnation, through the mother;

(*b*) At the moment of impregnation, through the father;

2. In the embryo or in the fetus by trans-placental infection.

A. INFECTION OF THE OVUM

If this infection is possible, it would seem to be exceptionally rare, because, as Virchow observed, an ovum containing tubercle bacilli loses its germinating properties and does not arrive at maturation. Meanwhile Maffucci[1] inoculated 18 hens eggs with avian bacilli and by incubating succeeded in obtaining 9 chicks, however delicate and of small frame. One died on the twentieth day, a second on the thirty-second day, others successively on the fortieth, forty-seventh and sixty-eighth day, etc., up to $4\frac{1}{2}$ months after hatching. At autopsy all of them showed tuberculous lesions.

Baumgarten[2] repeated this experiment. With a dozen eggs he obtained only two chicks, which died, one at four months, the other at four and one-half months, and which were likewise found tuberculous.

Obviously it does not follow that the infected human egg behaves necessarily as does that of the chicken. In fact no direct comparison is possible, since artificial infection of the hen's egg by direct introduction of bacilli into the egg white or yolk, more or less distant from the ovule, corresponds in reality to a placental infection (Milchner,[3] Weber and Bofinger,[4] Koch and Rabinowitsch[5]).

The possibility of carrying the virus into the healthy ovum by the fecundating sperm of the tuberculous father is likewise very

[1] Centralbl. f. Path. u. path. Anat., 1894, **5**, 1.

[2] *Die Bekämpfung der Tuberkulose*, Leipz., 1904, Hirzel.

[3] Beitr. z. klin. Med., Festschr. Senator, 1904.

[4] Tuberk.-Arb. a. d. k. Gsndhtsamte, 1904, H. 1, 83.

[5] Virchow's Arch., 1907, **190**, Beih., 246.

doubtful, although some writers think that they have been able to demonstrate it by experimentation. Moreover it is always very difficult to fecundate healthy females by tuberculous males, whereas the reverse is relatively easy, and the majority of experiments described as having been successful are at fault because sufficient precautions were not taken to avoid all risk of extra-uterine contamination.

F. Friedmann,[6] in a series of rabbits, injected a few drops of a suspension of human and bovine cultures into the vas deferens, and then paired the animals with healthy females. The latter were killed after 7 days. The one-week-old embryos were all found to contain bacilli.

On the other hand, if a suspension of bacilli was deposited in the vagina of females immediately after copulation with healthy males, the embryos developed normally and remained free from infection, although infection developed in several of the mothers. Seige[7] performed similar experiments and obtained the same results.

Prior to this, Gaertner[8] had introduced bacilli directly into the testicles of 22 rabbits and 21 guinea pigs which, on being coupled with healthy females, produced 29 young rabbits and 45 young guinea pigs. None of these offspring were found infected, although several of the females (about 1 in 5) were contaminated by the coitus, and became tuberculous.

It is a well-established fact that when tuberculous lesions exist in the seminal vesicles and in the testicles, secretions from these organs may contain bacilli; Jani,[9] Sirena and Pernice, Spano,[10] Jackh,[11] Nakarai, Dobroklowski,[12] and others have published many observations which permit of no doubt on this point. The sperm from such subjects is fairly often found to be infectious for animals into which it is inoculated. And yet all clinicians know that it is rather rare that a testicular tuberculosis in the husband leads to a primary infection of the genital organs of the wife. The observations published by Glockner and by Sellheim in this regard are scientific curiosities.

[6] Virchow's Arch., 1905, **181**, 150.
[7] Arb. a. d. k. Gsndhtsamte, 1904, **20**, 139.
[8] Ztschr. f. Hyg., 1893, **13**, 101.
[9] Virchow's Arch., 1886, **103**, 522.
[10] Rev. de la Tuberc., 1893, **1**, 31.
[11] Virchow's Arch., 1895, **142**, 101.
[12] Rev. de la Tuberc., 1895, **3**, 195.

It has been possible to prove in certain families that children issued of a healthy mother and of a father affected with tuberculous epididymitis were born and remained perfectly free from the infection. But there is nothing in the medical literature to prove infection of the ovum through a urogenital tuberculosis on the paternal side.

We are therefore justified in concluding, with Grancher and Hutinel,[13] with G. Kuss, and with Landouzy, *that there exists no positive evidence to prove that an infant may be procreated tuberculous by its father.*

B. TRANSPLACENTAL INFECTION

It is only when the fetal-placental circulation has replaced the first or primitive circulation, that tuberculous infection of the mother may transmit itself to the fetus, that is to say toward the end of the third month of gestation for the human race. Again, this latter event is possible only under exceptional circumstances, since the healthy placenta is a perfect filter; it allows leucocytes and bacteria to pass only when it is itself the seat of tuberculous lesions,—and this is extremely infrequent, or else in the course of certain febrile affections, such as variola, measles, scarlet fever, typhoid fever, malaria, or a bacillemia.

Experimentally, the passage of tubercle bacilli through the placenta, denied in the past by Cohnheim, has been realized by a few workers, in particular Landouzy and Hipp Martin,[14] later by de Renzi, Cavagnis,[15] Gaertner;[16] but many others, among whom I would cite Grancher and Straus, Von Leyden,[17] Nocard,[18] Wolff,[19] Jaquet, Baumgarten, Cornet,[20] Sanchez-Toledo,[21] and Vignal,[22] have failed completely.

[13] Internat. Congr. on Medicine, 13th. Paris, 1900;—Semaine méd., 1888, p. 297.

[14] Rev. de méd., 1883, **3**, 1014.

[15] Atti del Inst. Veneto, 1885/86, No. 4, 1145.

[16] Ztschr. f. Hyg., 1893, **13**, 101.

[17] Ztschr. f. klin. Med., 1884, **8**, 375.

[18] Arch. de méd. expér., 1889, **1**, 511 (quoted by Toledo); Ztschr. f. klin. Med., 1884, **8**, 386.

[19] Virchow's Festsch., Vol. iii.

[20] *Die Tuberkulose,* Vienna, 1907, Hölder.

[21] Arch. de méd. expér., 1889, **1**, 503.

[22] Internat. Congr. on Tuberculosis, Paris, 1891.

In order to most surely accomplish infection of the fetus through the maternal blood, Gaertner injected intravenously from 0.5 cc. to 2 cc. of culture into 10 healthy rabbits, which furnished him with 51 fetuses. Only 5 of the latter, or 10 per cent, were found tuberculous. In no instance was the entire litter infected.

The same experiment, repeated with mice tubercularized by the direct inoculation of a drop of culture into the trachea, gave similar results. Of 18 litters, comprising in all 74 young, 9 were infected.

On the other hand, Nocard injected healthy guinea pigs with the pulp obtained by grinding 40 fetuses produced by 4 tuberculous rabbits and 8 tuberculous guinea pigs. Not one of the inoculated guinea pigs developed tuberculosis.

The attempts of Grancher and Straus, with 14 fetuses, and those of Sanchez-Toledo, with 65 fetuses from infected mothers, were also completely negative. And so were those of Vignal, who used the liver and spleen of 11 human fetuses and 17 placentas from phthisical women.

It must be acknowledged therefore that, although fetal infection by way of the placenta is possible, it is at least very rare and as a factor in the propagation of tuberculosis is of very little importance (Lehmann,[23] Schmorl and Geipel[24]).

It seems however that, in cattle, this mode of contagion is more frequent. Siegen, Kockel and Lungwitz, and Nocard have reported it on several occasions. The greater frequency results perhaps from the fact that, in cows, the tuberculous infection remains quite often localized in the uterus or pelvic glands. The bacilli then break through the barrier of the placenta, "because necrotic lesions exist to make the breach."

Lenenberger contends that, in the human race, infection of the new born is brought about particularly *during labor*, through placental ruptures caused by violent contractions of the uterus. If bacillemia is present in the mother—and we know that such is the case fairly frequently in phthisis with cavities—the bacilli may pass directly from the maternal blood into the torn fetal vessels. The indication would then be, observes Landouzy,[25] to immediately ligate the cord in the new born of phthisical mothers, in order to avoid inoculation of the fetus before delivery.

[23] Berl. klin. Wchnschr., 1894, **31**, 601; 646.
[24] München. med. Wchsnchr., 1904, **51**, 1676.
[25] Internat. Congr. on Tuberculosis, 9th, Brussels, 1910.

Fetal infection thus brought about, either before or during labor, produces at times a tuberculosis with miliary tubercles localized primarily in the liver or scattered throughout the organism, or again a bacillosis without tubercles (Landouzy) and recognizable only after the death of the fetus by the inoculation of blood, or of pieces of liver or other viscera.

Max Zarfl[26] for example, gives the history of an infant whose mother died of phthisis three months after its birth and who reacted positively to tuberculin (cuti-reaction) from the age of 17 days. This infant died on the fifty-second day, with all the signs of a generalized lymphatic and blood infection. Almost all of its lymph nodes contained caseated tubercles, especially those of the intestinal cavity and those surrounding the portal vein. The bronchial glands were likewise involved, but less severely. It seems indeed that we have to do, in this case, with an infection brought about during labor or after birth, since the infant lived 12 days in contact with the mother, being nursed by her.

Clinicians have shown that congenital tuberculosis with formation of early-stage tubercles is rare. It involves the lungs but exceptionally and, in any event, only secondarily. The liver and spleen are the first organs to develop lesions. Through the glands of the liver hilus, the infection later reaches the mediastinum, the viscera of the thoracic and abdominal cavities, the serous membranes and occasionally even the bone-marrow.

Under certain circumstances, as Baumgarten claims, and as Liebermeister, Lannelongue, Maffucci, Landouzy, and Hutinel also admit, the fetal infection may be so slight as to produce only non-evolutive lesions, capable of remaining latent for a longer or shorter period and of developing only at a later date under the influence of other accidental infections (measles, scarlet fever, whooping cough, etc.). Here, at least, we have an hypothesis which seems indeed to be confirmed by certain clinical and autopsy findings, whose correctness, however, it has not yet been possible to establish by experiment.

C. HEREDITARY DYSTROPHIES.—SPECIFIC PREDISPOSITION

Many writers have called attention to the frequency of early death and congenital malformations in children of tuberculous parents.

[26] Ztschr. f. Kinderheilk., 1913, 8, 370.

"Some time ago," wrote Landouzy in 1891,[27] "I demonstrated, on the basis of a number of facts collected from the children's service at Tenon Hospital, that it was not at all exceptional to see tuberculous women and the wives of tuberculous husbands, after a first tuberculous child, have a whole series of pregnancies terminating before term or ending with the birth of sickly infants. The babies are delicate, below weight, undersized, and either succumb to marasmus a few weeks after birth, or to tuberculosis in the course of the first year. Now and then they die at the time of weaning or of dentition, most often without apparent cause. . . . . Their death is then classed under the heading of congenital debility."

Many of these infants present what Landouzy calls *general or partial dystrophies*, represented by *infantilism* or *nanism*, by *disturbances of nutrition* which Charrin[28] in particular has studied, by *cardiac or vascular lesions, pure mitral stenosis* (Potain, Hanot, P. Teissier), *pulmonary stenosis, arterial aplasia* and *arterial nephritis* (R. Moutard-Martin and Bacaloglu, Mosny)[29] or by *nervous stigmata* (hysteria, epilepsy, mental weakness, chorea, etc.).

These dystrophies have been reproduced experimentally by Landouzy and Hipp Martin, by Charrin, Robelin,[30] Gley, by Riche, Nattan-Larrier, G. Delamarre, Maffucci, Arthault de Vevey, Carrière, and others.

But we are indebted above all to Landouzy and Loederich[31] for the knowledge of certain facts which to their minds demonstrate the influence of tuberculous poisons on the pathogenesis of cardio-arterial malformations and the influence of tubercle bacillus infection on certain congenital bone lesions (curvatures, club foot, etc.) and on what it has been agreed to call by the more general term of *hereditary predisposition to tuberculosis*.

There has been much discussion in recent years, at the congresses devoted to the study of tuberculosis, as to whether children *born of tuberculous parents* but *not infected themselves and not harboring bacilli at birth,* often bring with them into the world organic

[27] Rev. de méd., 1891, **11**, 410; 721.

[28] Compt. rend. Acad. des sci., 1898, **127**, 332.

[29] Rev. de la tuberc., 1898, p. 297; 1899, p. 311; 1901, p. 301—Ann. d'hygiène publique et de médecine légale, 1902, p. 289.

[30] Thèse, Paris, 1902.

[31] Internat. Congr. on Tuberculosis, 9th, Brussels, 1910.

deficiencies which render them more susceptible to infection than children born of healthy parents. This is one of the most important problems in determining the direction to be taken by the anti-tuberculosis campaign.

The great natural susceptibility of cattle to tuberculosis is well known, and they might have been used to furnish much valuable information in this respect. But no one up to now has thought of taking calves, *born tuberculosis-free*, and keeping them long enough isolated from their tuberculous mothers, and shielded from any natural contagion, to measure later their resistance either to artificial infection or to that rendered possible through confinement with other animals, as compared with the resistance of other calves born of normal cows.

The only positive findings which we possess at the present time are those collected in the abattoirs of the large cities and on certain farms. They all prove the extreme rarity of tuberculosis in calves less than 6 months old and confirm the fact brought out more than 25 years ago by Bang, later by Nocard, by Ostertag, and by Hutyra, that *calves separated from their tuberculous mothers at the time of birth, fed with the milk of healthy cows and shielded from any contact infection, remain free from tuberculosis indefinitely.*

Unfortunately all of these experiments, carried out with animals whose normally brief life is still further shortened by economic necessities, give us but imperfect information on this question which most deeply concerns humanity, namely the apparent special aptitude possessed by children born of tuberculous parents to contract tuberculosis. Thus in order to enlighten ourselves on this point, we are obliged to resort principally to clinical observation and to animal breeding.

The latter, as my collaborator C. Guérin[32] has written, tends to confirm the proposition long since upheld by Landouzy, relative to the apparent predisposition to tuberculosis possessed by blond individuals with auburn silky hair and pale freckled skin of fine texture.

It seems evident, according to C. Guérin,—and this also is the opinion of Dechambre (of Alfort),—that certain races of cattle with light colored coat and hide contract tuberculosis more easily than

[32] Internat. Congr. on Tuberculosis, 9th, Brussels, 1910.

those of other races placed in the same stables. It would seem even that these particularly susceptible animals (race of Landes in France, Durham in England), on being crossbred, transmit a part of their susceptibility to the offspring.

Certain analogous facts, as to whose importance we shall have to return in another chapter (XL), are observed in human races. It is claimed for example that certain people of Oceania, particularly the Tahitians and also the blacks of Senegal and Central Africa, among whom tuberculosis is a matter of recent importation, possess an extreme degree of susceptibility to infection by the tubercle bacillus. The disease assumes grave forms and develops rapidly, causing terrible ravages among them. Inversely, in all the large centers of population of the United States, as in those of Europe and of North Africa, the mortality from tuberculosis is much lower in the Jewish race than in the rest of the population. In the whole of the United States only 37 die from tuberculosis per 1000 deaths among the Jews, while among the population as a whole 138 of each 1000 deaths are due to tuberculosis (Fishberg).

The opinion is therefore fairly widespread that a more or less special susceptibility or resistance to tuberculosis exists in certain races of man and animals. But, up to now, it has never been proved that this susceptibility or resistance,—granted that it exists,—results from a specific hereditary impregnation by secretory products of the bacillus. It would seem indeed to be rather a matter of structure or special arrangement of lymph glands and lymphatic apparatus.

Tuberculin impregnation of a tuberculosis-free infant by the tuberculous mother is an hypothesis not borne out by experimentation. In fact, we know, on the one hand that tuberculin is a weakly and slowly dialyzable toxin, and on the other hand that *infants born of infected mothers*, when *themselves free from congenital lesions*, are completely insensitive to it. Finally, it is easy to prove, as I have by many experiments, that the lethal dose of tuberculin for young tuberculosis-free animals is the same regardless of whether the latter are born of healthy or tuberculous mothers.

D. CONTAMINATION AFTER BIRTH.—FAMILY CONTAGION

If we except some altogether rare cases in which infection by the tubercle bacillus obviously took place before birth, it may be said that this infection as a general rule occurs only after birth.

Further along we shall see (*Chapter XXV*) that, under certain circumstances and especially in certain countries (England and Ireland), infection of infants occurs through the milk of tuberculous cows with which they are fed. But it is incontestable that, everywhere and always, it is *family contagion*, and more often *contagion by the phthisical mother*, which plays the chief rôle.

Dörner[33] carried out an investigation in the Duchy of Baden and found that among children dying from tuberculosis in the course of the first two years of life, 33.5 per cent had phthisical mothers. Only 14.7 per cent had been infected from the father.

This preponderance of contagion through the mother or by the nurse is being constantly affirmed. It stands forth—we shall have occasion to return to this subject (*Chapters XXXVI and XL*)—in all the statistics of tuberculin reactions in young children. It is revealed also in the observations of clinicians. Comby,[34] for example, has published a whole series of facts which demonstrate it, and he proved that infants born of phthisical parents escape tuberculosis to the extent of 97 per cent if guarded from infection and placed in the country under hygienic conditions, while 50 per cent die if left exposed to family contagion.

Bang (of Copenhagen), and Nocard later, have shown likewise that young calves born of tuberculous cows remain perfectly healthy and never react to tuberculin if care is taken to separate them from their mothers from birth and to feed them with sterilized milk or with that from cows definitely free from tuberculosis.

Herbert G. Lampson[35] made an extensive study of the distribution of tuberculosis among poor families of Minneapolis (Minnesota, U. S. A.). He found that 67 per cent of children born of parents with *open lesions* became tuberculous at an early age. The proportion of those affected in families where one or more members had only *latent lesions* was not above 22 per cent while in *tuberculous-free* families it was only 2.5 per cent.

In almost all tuberculous nurslings family contagion appears evident. It may be brought about by the mother or by the wet nurse who has the bad habit of moistening her breast with saliva before the nursing, or who tastes the porridge with a spoon to assure that

[33] Beitr. z. klin. d. Tuberk., 1911, **20**, 1.

[34] Arch. des mal. des enfants, 1905, **8**, 641.

[35] Bull. Univ. of Minnesota, 1913.

the temperature is correct; by the mother who wipes the baby's eyes and mouth with her handkerchief moist with bacillus-containing sputum; by kisses upon the mouth; a little later, when the child is learning to take his first steps, by the dirty fingers which he sucks after creeping over the sputum-soiled floor (*tuberculose des enfants touche à tout*).

All of these causes and opportunities for infection amass themselves. Is it astonishing then that the number of virulent bacilli thus absorbed each day in small doses, at times in massive doses, end by causing lesions which choke the glandular filters?

The preceding considerations oblige us therefore to conclude:

1. That *hereditary transmission* of the tubercle bacillus manifestly occurs under certain circumstances and that it then results from *an intra-uterine infection consequent to tuberculous lesions of the placenta* or from a blood infection produced *at the moment of birth.* But *infection after birth, in the infected family, is a factor infinitely more important.*

2. That children, born free from tuberculosis and of parents seriously affected with the disease, present frequently the stigmata which are characterized under the denomination of *hereditary dystrophies.*

These young subjects, placed in an environment of infection, readily contract tuberculosis and resist it poorly by reason of their general debilitated condition; but they may be saved by protecting them against opportunities for infection.

PART TWO

Experimental Tuberculosis and Tubercle Bacillus Infection in Animals

CHAPTER XX

DIFFERENT MODES OF INOCULATION AND OF EXPERIMENTAL TUBERCULOUS INFECTION

A. EXPERIMENTAL TUBERCULOUS INFECTION BY SUBCUTANEOUS INOCULATION OF VIRULENT MATERIAL.—CONDITIONS OF EXPERIMENTAL INFECTION.—INFLUENCE OF THE NUMBER AND VIRULENCE OF THE INFECTING BACILLI

If there be introduced under the skin of the thigh of a susceptible animal, such as the guinea pig or monkey, either a small quantity of sputum from a phthisical patient, as was done by Villemin in 1865, or tubercle bacilli from a pure culture of human or bovine origin, the immediate results of this inoculation are nil. In a short time a mild local inflammation with a little edema makes its appearance. It is not until after 6 to 10 days that palpation of the groin, corresponding to the point of inoculation, reveals an enlarged hard gland rolling under the fingers like a foreign body. This gland at first is the size of a hemp seed, but before long increases to that of a small nut. The animal remains apparently well and may even gain in weight. Then about the third or fourth week it begins to grow thin; the hair loses its gloss and tends to bristle, the ribs become visible, the flanks fall away and respiration is accelerated. The temperature, normally between 38 and 39°C. in the guinea pig, is now constantly above 39.5. Emaciation becomes more and more pronounced until at last by the eighth or tenth week, sometimes a little earlier or later depending on the dosage of inoculated virus, extreme cachexia is followed by death (*fig. 13*).

Autopsy now reveals the whole series of multiform lesions which characterize generalized tuberculosis. The much enlarged initial gland is surrounded by edematous tissue and on section is found filled with a creamy cheesy pus, homogeneous and free from odor. The abdominal cavity contains more than the normal quantity of fluid. The liver, which may be twice its normal size, shows surface bosses and numerous rounded or irregular masses of a yellowish

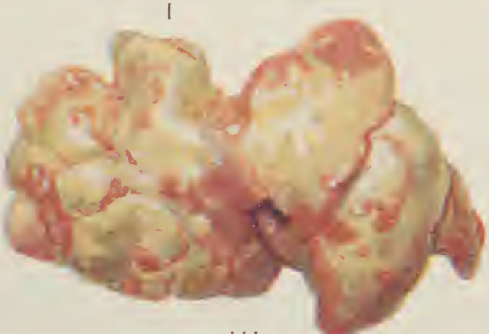
PLATE XII

1. Generalized tuberculous infection in a guinea pig after subcutaneous injection of $\frac{1}{100}$ of a milligram of bovine tubercle bacilli into the left thigh (death on the 45th day).

2. Tuberculous spleen and liver in a state of cirrho-fatty degeneration in a guinea pig inoculated subcutaneously every other day for three weeks with 5 to 8 tubercle bacilli of the human type. The animal was killed after 240 days.

I

II



III

white color and often containing pus. The spleen, 6 or 8 times its normal size, or even larger, is like a mosaic of yellowish grains which vary in size from the head of a pin to that of a lentil and which contain cheesy pus. The kidneys are pale; the supra-renal capsules are much enlarged and have a bluish color with an effect of mother-of-pearl.

On opening the thoracic cavity, disseminated tubercles are seen everywhere studding the two lungs, but generally more abundant in the posterior lobes. Some of these tubercles are very small and grayish, while others are as large as the head of a pin with a white

Fig. 13. Technique of Subcutaneous Inoculation, into the Thigh of the Guinea Pig, for the Diagnosis of Tuberculosis

central point and a transparent hyalin zone at the periphery. The neighboring pulmonary tissue is partially hepatized and red. The tracheo-bronchial lymph nodes form a large conglomerate mass which encloses the trachea at the point of bifurcation. The node centers are softened and caseous. Finally, at each side of the sternum, on its diaphragmatic wall, are other glands smaller in size but equally caseous (*see Plate XII, 1*).

On staining with Ziehl, the contents of all these lesions show microscopically an abundance of tubercle bacilli, some free, others contained within the lymphatic cells.

The rapidity with which tuberculous infection becomes generalized and develops, in the guinea pig, varies within wide limits according to the dosage and virulence of the infecting bacilli.

Guinea pigs never, or almost never, contract tuberculosis spontaneously. In raising them, even in places connected with laboratories, it is altogether exceptional to find any of these animals infected. By simply living together with artificially infected animals in cages or in pens, they very seldom infect themselves.

This apparent immunity to natural infection is probably due to the fact that the *solid dejections of the guinea pigs do not soil their food,* and that the latter, made up exclusively of roots, bread or bran, cannot be for them a source of infection.

On the other hand, guinea pigs manifest an extreme degree of susceptibility when infected artificially by the various methods. Of all the animals commonly used in the laboratories they are, next to the monkey, the most highly susceptible. It is therefore chiefly to them that we must have recourse for the study of experimental tuberculosis and for inoculations having as an object the establishment of a diagnosis.

The rabbit is much less susceptible, especially to human tubercle bacilli, as will be seen later. Yet this animal may quite often be used to advantage, particularly when it is necessary to determine the type of a culture of bacilli, whether human, bovine or avian, or the nature of a lesion in another animal.

Like the guinea pig, and for the same reasons, the rabbit contracts tuberculosis very rarely, even when caged with artificially infected animals, and never contracts it spontaneously when being bred.

When the *virulence* of tubercle bacilli is to be studied experimentally inoculations must always be made with *a strain of bacilli obtained by planting tuberculous organs or material directly* upon artificial media, or at least with a culture made *after not more than one passage through the guinea pig.*

In every experiment the weight of the bacilli employed must be definitely determined. In order to make this calculation, a small quantity of culture is taken up with a sterilized platinum spatula and weighed upon a square of sterile filter paper (previously weighed) on a watch crystal. *Ten milligrams,* for example, are taken.

These bacilli, after being blotted on the filter paper, are put in a sterile agate mortar and carefully suspended in sterile 1 to 10,000 solution of sodium carbonate, added drop by drop with a pipette from the beginning. The suspension is more homogeneous and stable if two or three drops of sterile ox bile or a similar quantity of fresh yolk of egg are added early in the mixing.

Clumps are to be avoided and in order to remove them the suspension should be poured into a sterile cone shape vessel and allowed to settle for a half hour before decanting the opalescent supernatant fluid. Only the latter should be used to prepare the desired dilutions.

In calculating dilutions, the average number of bacilli contained in *1 milligram* of culture on solid media (glycerin potato for example) is used as a basis. In my experience this figure is roughly 40 millions. Therefore *in one ten-millionth of a milligram there should be four bacilli,* each of them weighing about 2.5 hundred millionths of a milligram.

In a large number of trials I have found that the average number of bacilli capable of infecting a young guinea pig, of 250 gms. for example, varies with the source of the culture and bears a close relationship to the virulence. *The degree of virulence of a culture therefore may be measured in terms of the number of bacilli capable of infecting a 250-gm. guinea pig by subcutaneous inoculation into the thigh.* Older guinea pigs are more resistant and more bacilli are required to infect them. The tests should be made on several animals of approximately the same weight if reasonably accurate figures are to be had. The results differ if the bacilli, instead of being inoculated subcutaneously, are introduced into the peritoneum, for example, or through the digestive tract.

With very virulent cultures a minimum of *10 bacilli,* more often *50 bacilli,* are required to infect by the subcutaneous path. Certain writers have claimed that a single bacillus is sufficient to transmit tuberculosis but this statement is not accurate. I have, however, shown in the course of many experiments in collaboration with Bruyant reported further along (*Chapter XXXVIII* and *XXXIX*), that if *4 bacilli per day,* for example, are injected into guinea pigs subcutaneously, at different points of the body, over a period of 10 days, infection unfailingly results, whereas a single injection or two injections of 4 bacilli are absolutely innocuous.

Thoeni and A.-C. Thayssen[1] (of Bern) have also studied the effects of a single inoculation of a very small number of bacilli taken from cultures of known virulence. They found as I did that guinea pigs do not contract tuberculosis when only a few isolated bacilli

[1] Centralbl. f. Bakt., 1916, 77, 308.

are introduced. They injected 19 guinea pigs with from 10 to 76 bacilli and found that only one (inoculated with 71 bacilli) showed tuberculous lesions.

In other susceptible animals such as rabbits, monkeys or cattle, similar relationships obtain. *Infection therefore is the resultant of several factors of which the most important are: virulence* and *number* of the infecting bacilli introduced *simultaneously* into the body, *repetition* of infections *at shorter or longer intervals, path of infection* (subcutaneous, intraperitoneal, enteral, etc.), and finally the *degree of susceptibility* of the animal species.

These facts, well established experimentally although generally little known, are of very great importance. The reader must keep them well in mind in order to understand what will be said further on as to resistance to infection and as to the processes of immunization against tuberculosis.

B. DIFFERENT MODES OF EXPERIMENTAL INOCULATION OR INFECTION OF THE GUINEA PIG AND RABBIT

Experimental infection of laboratory animals, and particularly of the guinea pig, may be accomplished in different ways. In addition to the already discussed subcutaneous route, the pathways most commonly employed are:

a. The intraperitoneal.
b. The intravascular or intracardiac.
c. The intracranial or intraspinal.
d. The eye.
e. The digestive tract.
f. The rectum.
g. The bladder.
h. The respiratory.
i. The transcutaneous.
k. The intramammary.
l. Finally, and exceptionally, bacilli have been inoculated directly into the gall-bladder or into an intestinal loop after laparotomy, or else into the pleura or into the interior of a joint.

A knowledge of the anatomy of the lymphatic system in the guinea pig and rabbit is indispensable if one is to follow the course of the virus in the body of these animals. *Figures 14* and *15* may be consulted to advantage.

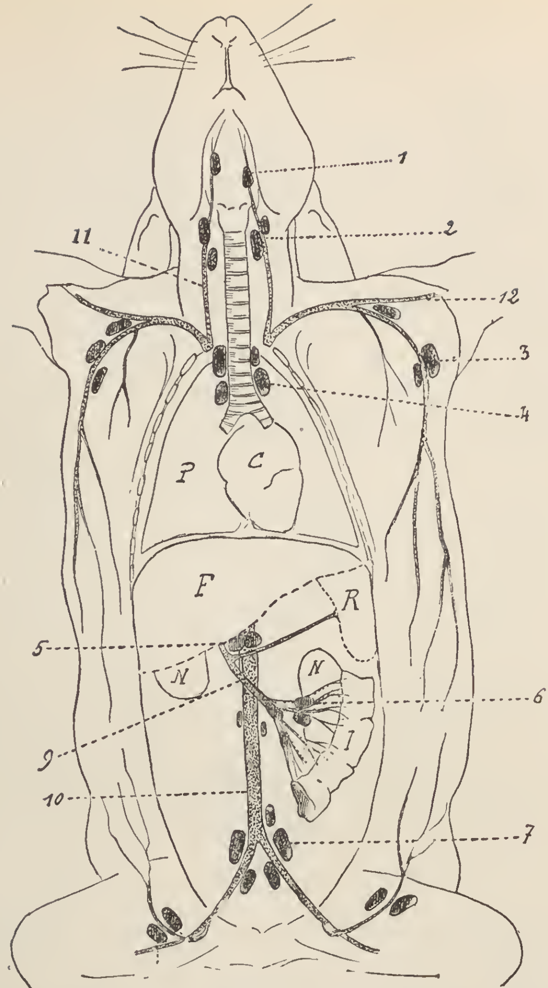


FIG. 14. SCHEMA OF THE LYMPHATIC GLAND SYSTEM IN THE RABBIT
(DRAWN BY L. BRUYANT)

1. Sub-maxillary glands
2. Retro-pharyngeal and cervical glands
3. Axillary glands
4. Tracheo-bronchial glands
5. Periportal glands
6. Mesenteric glands
7. Sub-lumbar and iliac glands
8. Inguinal glands
9 and 10. Inferior vena cava
11. Jugular vein

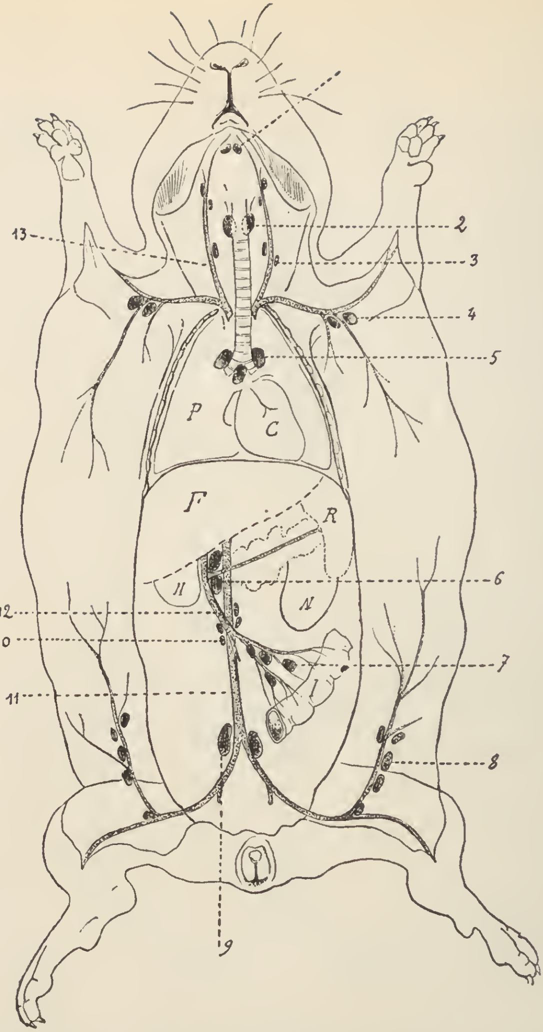


FIG. 15. SCHEMA OF THE LYMPHATIC GLAND SYSTEM IN THE GUINEA PIG (DRAWN BY L. BRUYANT)

1. Sub-maxillary glands
2 and 3. Cervical glands
4. Axillary glands
5. Tracheal glands
6. Periportal glands
7. Mesenteric glands
8. Inguinal glands
9. Iliac glands
10. Portal vein
11 and 12. Inferior vena cava
13. Jugular vein

a. Intraperitoneal inoculation

The guinea pig is held by an assistant or fastened upon its back on a special apparatus and the hair removed with a pair of curved scissors over a surface of 2 or 3 square centimeters, either a little to the right or to the left of the umbilicus.

This surface is cleaned with a mixture of equal parts of absolute alcohol and ether, or painted with tincture of iodine. After waiting a few moments the needle is quickly introduced for about 1 centimeter vertically through the skin into the peritoneal cavity. One should be sure that the point of the needle is freely movable in all directions; injection is gently made and the needle quickly withdrawn The small wound is again washed with alcohol-ether or tincture of iodine and the animal set free.

This method of inoculation should be employed only with suspensions of cultures or of fresh tuberculous organs. It should never be used to detect bacilli in sputum or fecal matter, urine, pus, or decomposed cadaver material since fatal peritonitis may result from the presence of other bacteria.

Intraperitoneal injection is usually followed by rapid extension of tuberculosis to the abdominal viscera, then to the thoracic viscera, with engorgement of the retro-sternal glands followed by their caseation and the formation of a mass of tubercles in the omentum.

If bacilli of moderate virulence are injected in a dosage of 0.1 mgm. (weighed in fresh state) death generally ensues in 3 to 4 weeks.

b. Intravascular and intracardiac inoculations

Infection of the guinea pig or rabbit by the blood stream is always very severe, even though very small doses of virus be used. At the same time bacilli of the *human type,* as we shall see later, are not very active in the rabbit, while those of bovine origin are more quickly fatal. With *10 mgms.* of a fresh culture of *human bacilli* of moderate virulence, a rabbit of 2 kilograms succumbs only after 3 to 4 months, often longer, whereas a dose of 0.1 mgm. of *bovine* bacilli suffices to produce death with lesions of generalized miliary disease in less than 2 months.

Intravascular inoculation can be made very easily in the rabbit into the marginal ear vein, which can be seen through the skin and lies against hard resistant connective tissue. The vessel is pierced

parallel to the skin surface with the needle directed toward the base of the ear.

One can easily infect the blood stream of the rat and mouse (method of Weidanz-Trommsdorf[2]) through the two superficial, readily accessible veins at the base of the tail. For mouse injections a very fine needle should be used similar to that employed by dentists for the injection of anaesthetic solutions into the gums. As much as 2 cc. of fluid may be introduced into the vein of a mouse. The animal being held by an assistant, the operator grasps the tail

Fig. 16. Technique of Intravenous Inoculation into the Guinea Pig

with the left hand, with the base on his index finger, and inserts the needle parallel with the vessel. If the point of the needle is withdrawn slowly, even the smallest drop of blood may be avoided.

In the guinea pig there is no superficial vein which is readily accessible. The injection must be made into the jugular vein previously exposed. Immediately after injection the vein is either ligated or the flow of blood stopped with a small clamp which is left in place a few minutes before closing the wound (*fig. 16*).

In the rabbit and in the guinea pig it is often preferable to infect the blood stream by direct injection into the carotid artery which

[2] Arb. a. d. k. Gsndhtsamte, 1909, **32**, 568.

can be easily isolated and entered between two loops of thread. The ligature is drawn tight immediately after the fluid has been injected, and over the needle itself before the latter has been withdrawn.

Intracardiac injection is much more to be recommended, since it can be done without incision. With a little practice and with care to locate the point of puncture according to the technique first described by Pagniez,[3] then by A. Raybaud and Ed. Hawthorn,[4] and better still by Ch. Nicolle and E. Ducloux,[5] a syringe needle is introduced with a quick thrust a little obliquely from below upward and from before backward into one of the ventricles of the heart. If the blood flows out in spurts the tip of the syringe is quickly adapted to the needle, the bacterial suspension gently injected, and the needle abruptly withdrawn.

The best point for entering the heart is, in the rabbit, in the third left intercostal space, three millimeters outside the border of the sternum; from here the needle enters the right ventricle. In the guinea pig the site of election is along the left border of the sternum 8 to 10 mm. above the apex of the angle formed by the xiphoid cartilage and the last costal cartilage articulating with the sternum. The needle, which should be introduced to a depth of 15 to 17 mm., thus enters above the next to last chondro-sternal articulation and enters the left ventricle. A little higher it reaches the auricle; lower down it passes through the diaphragm and pierces the liver. The needle should be pointed slightly inward toward the median line.

This small operation is entirely harmless. It may be carried out several times in the same animal, as is done in collecting blood for fresh complement for the Bordet-Gengou reaction.

Tuberculous infection by the intracardiac route, when produced with very small doses or with attentuated bacilli, sets up a variety of lesions in all the viscera, with curious localizations at times in the kidneys, testicles, ovaries or joints. These forms of tuberculosis are altogether similar to those observed quite often in cattle or in man.

[3] Thèse, Paris, 1902.

[4] Compt. rend. Soc. de biol., 1903, **55**, 815.

[5] Ibid., 1903, **55**, 904.

c. Intracranial and intraspinal inoculations

Intracranial infection may be realized in two ways: the first consists in injecting the virus under the dura mater or into one of the cerebral hemispheres. This is easily reached if a small incision is made in the skin over the vertex in a transverse line connecting the two posterior eye commissures and a little hole drilled in the cranium. The orifice having been carefully made a little to the right or left of the median line,—in order to avoid the superior longitudinal sinus,—the point of the needle is introduced to a depth of 4 to 5 mm. and a quantity not exceeding 4 to 5 drops gently injected.

The other method, which is at once neater and less dangerous, is that of post-orbital injection which we introduced into France after seeing it commonly performed in American laboratories. The technique is as follows:

With the head of the animal—guinea pig or rabbit—firmly held in the horizontal position, the syringe needle is grasped by its butt and introduced with a sort of rotary motion, from without inward and from before backward, into the depth of the orbital cavity, along the internal surface of the eye-ball, and without wounding the latter. As soon as the needle point reaches well into the posterior part of the orbit a slight movement from below upward causes the needle to penetrate through the optic foramen to a point underneath the optic chiasm and without the latter being injured. The needle is known to be in place when it moves freely in the optic foramen without striking against any bony wall. The fluid is then injected and penetrates between the dura and pia mater to mix with the cerebrospinal fluid.

The needle, being quickly withdrawn, leaves no wound, nor does the operation cause the animal any harm.

This method of inoculation enables one to produce experimental meningitis which is quite typical and fatal in from 2 to 3 weeks.

Intraspinal inoculation is made by lumbar puncture, a steel needle being introduced from behind forward between the laminae of the next to last lumbar vertebra, into the spinal canal in which is the cauda equina. A preliminary skin incision is indispensable. It should be about 1 cm. long and over the spinous process of the 4th lumbar vertebra. The latter is accurately determined with the tip of the index finger of the left hand and the needle immediately introduced. After a few drops have flowed out to indicate that the needle is well in place, the syringe is connected and the infecting

material gently injected, not more than 0.5 cc. for the guinea pig and 1 cc. for the rabbit. The needle is then quickly withdrawn and the small cutaneous wound closed with a skin hook (agraffe) and touched with tincture of iodine.

d. Inoculation by the eye

This is carried out either by introducing the virus directly *into the anterior chamber of the eye* or by simple *instillation into the conjunctival sac.*

Inoculation into the anterior eye chamber is more convenient in the rabbit.

The eye-ball is held by means of mouse tooth forceps which seize a fold of the conjunctiva a little outside the upper margin of the cornea. The latter is now pierced very obliquely with a fine needle, care being taken not to wound the iris. After allowing a few drops of aqueous humor to escape, the syringe is adapted to the needle and the bacterial suspension gently injected. Only a small quantity is introduced in order to avoid too great intraocular tension.

If the needle is quickly withdrawn the small corneal wound closes immediately and the surface of the eye need only be washed with a little boiled water or 3 per cent boric acid solution.

This method enables one to observe the development of tubercles *in vivo* along the free margin of the iris, and to follow the infiltration of leucocytes leading up to giant cell formation.

As a general rule no change in the appearance of the eye-ball is observed during the first week after inoculation. After an incubation period, varying from about 12 days in the guinea pig to 15 to 30 days in the rabbit, the iris suddenly becomes much inflamed. Its free border is soon covered with grayish points which proceed to multiply over the whole surface and project into the anterior chamber. Becoming gradually more whitish they conglomerate and end by invading the cornea, which then becomes opaque and ulcerated. This whole process runs its course in 6 to 12 weeks. Excellent descriptions of such lesions will be found in the papers of Hänsell,[6] Karl Schuchardt[7] and F. Schieck.[8]

[6] Arch. f. Ophthal. (Graef's), 1879, **25**, 1.

[7] Virchow's Arch., 1882, **88**, 28.

[8] Veröffentl. d. R. Koch Stift. z. Bekämpf. d. Tuberk., 1913, H 5/6, 1.

Simple instillation of a drop of sputum containing bacilli (*fig. 17*) or of a culture upon the surface of the conjunctiva of guinea pigs, rabbits, monkeys or other mammals, represents a natural form of infection whose first apparent manifestation is an engorgement of the glands of the neck (Calmette, C. Guérin and V. Grysez).[9] The engorgement varies in intensity and rapidity according to the dose of virus instilled. Afterward the infection gradually involves the tracheo-bronchial glands, the lungs and the abdominal viscera. A local lesion of the eye itself is never observed under such circumstances (*see Plate V, 2*).

FIG. 17. TECHNIQUE OF LYMPHATIC TUBERCULOUS INFECTION IN THE GUINEA PIG, BY OCULAR INSTILLATION

e. Infection by the digestive tract

Properly speaking, infection by the digestive tract is the most *natural* form.

In young animals it usually produces primary lesions of the mesenteric glands and, in older animals, the various types of glandular and visceral tuberculoses which are observed with spontaneous infection (*fig. 18*).

The technique consists either in simply painting the inside of the cheeks with the infectious material, in mixing the bacilli or bacillus-containing matter with the food, or in introducing the bacilli or their

[9] Compt. rend. Soc. de biol., 1913, **74**, 310.

products directly into the stomach by means of a syringe and esophageal tube which, in the case of the guinea pig, is a small size rubber catheter. The tip of the latter is passed gently along the soft palate, an assistant holding the animal's head up and keeping its jaws apart with two flat bands in order to prevent biting of the tube. If respiratory movements continue to be regular and there is no special coughing effort, one may be certain of having passed the tube into the esophagus.

Fig. 18. Technique of Tuberculous Infection of the Guinea Pig by Ingestion through an Esophageal Catheter

f. Infection by the rectum

In order to prevent immediate expulsion of bacillary emulsions injected into the rectum, they should be carried as far up as possible (about 5 cm. in the rabbit) by means of a very small and very flexible rubber catheter.

Absorption of bacilli introduced into the rectum is much less certain than by the stomach. Tuberculins, on the other hand, are absorbed quite readily.

Rectal infection extends first to the sub-lumbar and mesenteric glands and afterward becomes generalized in all the abdominal and thoracic viscera.

g. Infection by the bladder

This method of infection enables one to follow the development of ascending lymphatic lesions beginning in the mucous membrane of the bladder. It is carried out by gently introducing a very fine soft Gaillard catheter into the urethra, to a depth of 3 cm. in the guinea pig. The catheter should be sterilized in formol and then dipped into sterile oil. As much urine as possible is allowed to flow out and the bacillary suspension then injected.

On killing the animals at various intervals after infection in this manner, there are found either more or less abundant and opaque granulomata, ulcerations or true caseous abscesses of the bladder wall. The infection extends always to the regional lymph glands, first to the sub-lumbar, then progressively to the whole lymphatic system along the ascending path, to the abdominal viscera, and to the lungs. M. Breton[10] (*Chapter XV, C*) found in his experiments that involvement of the tracheo-bronchial glands occurred as early as did the engorgement of the sub-lumbar glands. It was constant by the tenth day.

h. Infection by the respiratory passages

Inhalation of infectious dust, whether dry or moist, does not produce primary tuberculization of the lungs as readily as one would think. Consequently those using this method of infection have frequently been confronted with discordant results not always to be explained by differences of technique. These results have however been explained in Chapter IX.

Infection by inhalation may occur in any portion of the respiratory tract; nose, mouth, pharynx, larynx, trachea, bronchi, or pulmonary alveoli, and it is extremely difficult,—one might say impossible,—to confine it to any one of these segments. One may, however, avoid the outer segments (nose, mouth, pharynx, larynx) by injecting the virus with a syringe directly into the trachea or by blowing the material into the bronchial tree after tracheotomy.

As a rule the animals are fastened down on a special apparatus like that employed by Kuss and Lobstein in their anthracosis experiments.[11] The infectious matter, dry or moist, is blown in through

[10] Ann. de l'Inst. Pasteur, 1910, **24**, 820.
[11] Bull. méd., 1906, **20**, 911; 1046; 1059; 1071.

a small opening, care being taken to leave an aperture at the opposite end in order to avoid too much pressure. This aperture of course must be provided with a filter or attached to a wash bottle in order to prevent the escape of bacilli to the exterior.

Instead of a box, which is very difficult to keep tight and in which the suspended particles are very unequally distributed, we prefer the arrangement shown below (*fig. 19*).

The animal, guinea pig or rabbit, is fastened by its four feet upon a board, the head alone penetrating through a small opening in a rubber diaphragm and lying free in a glass chamber provided with

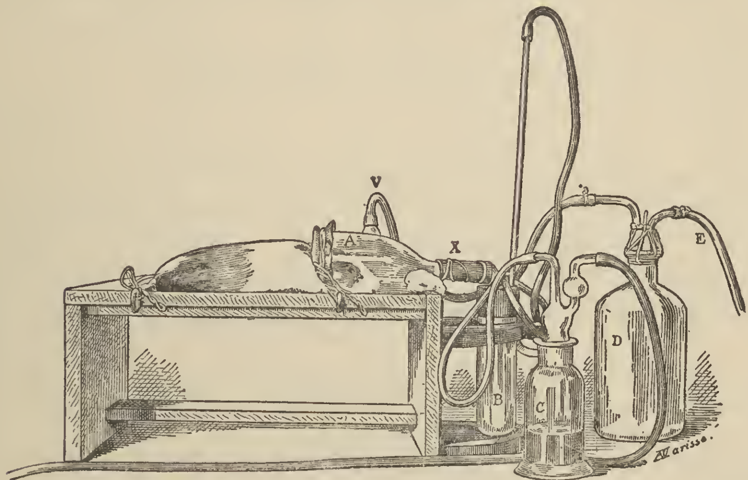


FIG. 19. APPARATUS FOR TUBERCULOUS INFECTION OF THE GUINEA PIG THROUGH INHALATION

A. Glass chamber with rubber diaphragm through which the head of the animal is introduced. The chamber has two apertures, one (*X*) for the entrance of the liquid spray, the other (*V*) through which the uninhaled and uncondensed excess of spray in the chamber is carried to the wash bottle *C* which contains a 1 in 4 dilution of sulphuric acid.

B. Büchner atomizer.

D. Pressure-regulating bottle, the cork of which has a valve arrangement.

E. Tube connecting with the apparatus for air compression.

two small tubes. Through one of them is introduced the infectious material mixed with air compressed by a small electric pump to which is attached a flask with a pressure regulating valve. The excess of material is carried away by the outlet tube, after bubbling through the sulphuric acid contained in the wash bottle.

With this apparatus, which is devoid of danger for the operator, one can easily control the number of bacilli in the air inhaled and at the same time count the respirations of the animals, both of which are impossible with the apparatus of Kuss as well as with that of Reichenbach employed by Karl Flügge and H. Findel.[12]

In a still simpler manner one may, like Chaussé, leave the animals at liberty in a more or less spacious room into which the infecting material is made to penetrate in the form of fine dry or moist particles. The latter, when sufficiently minute, that is to say when their diameter does not exceed 10 microns, may be borne to a distance of 3 meters (as can be proved in using colored material in a similar manner) but they remain for only a few seconds in suspension in the air unless the latter is kept in continuous motion.

Infection by the respiratory tract does not produce primary infection of the lung unless the number and virulence of the bacilli introduced into the pulmonary alveoli with the inspired air are such that these bacteria are capable of creating one or more local lesions. If the number of infecting organisms is few and if their virulence is low,—as is usually the case with dry bacilli,—they are phagocytized by the leucocytes, transported into the lymphatics and then into the blood circulation, are borne in the latter for a shorter or longer period to end finally by being eliminated from the body or by initiating the formation of a tubercle through a capillary or lymphatic embolus. Then lesions may be set up in organs other than the lungs, as after infection through the digestive tract, the mucous membranes or skin.

i. Transcutaneous infection

Tubercle bacilli may easily pass through the skin when the latter is the seat of an injury, even though this be very superficial such as might be produced by too close shaving or by pulling out the hair. Leucocytes may wander from the sub-epidermal lymphatic vessels and, ingesting the bacterial elements, bear them into the circulation. If the infection is a massive one or very virulent, they may create tubercles in situ or in the immediately neighboring lymphatic area; but if the bacilli are few in number or of low virulence, there may be no evidence of their passage left in the skin and they may not

[12] Ztschr. f. Hyg., 1907, 57, 104.

produce glandular, pulmonary or visceral localizations until much later.

The technique of transcutaneous infection is very simple: it consists in shaving or carefully epilating, in the guinea pig or any other animal, an area of skin over the upper part of the neck, so that the animal may not lick it. The culture or sputum or any other tuberculous material reduced to a fine pulp is then spread with a spatula over the freshly shaved or epilated surface.

k. Intramammary infection

In the lactating cow or goat this is accomplished by inserting a milking tube or flexible rubber catheter into the milk ducts, so that the infecting substance may be injected directly into the glandular acini.

Furthermore in the same animals, the syringe needle may be introduced into the glandular tissue at the base of the udder, as was done by Nattan-Larrier in the lactating guinea pig. A very rapidly progressive infection is thus produced. The bacilli multiply very fast and are found in abundance in the milk, and at the same time there is intense engorgement of the supra- or retro-mammary lymphatic glands.

l. Inoculation into the gall-bladder or peritoneum after laparotomy

In the guinea pig, and still more easily in the rabbit, the virus may be introduced directly into the gall-bladder exposed by operation. The animal should be completely anaesthetized to avoid any straining which might cause the intestines to be extruded from the abdominal cavity. Anaesthesia is best accomplished by intraperitoneal injection of chloral-morphine (1 cc. in the guinea pig, 2 cc. in the rabbit of a 10 per cent solution of chloral to which is added 0.5 per cent of morphine-hydrochlorate).

The technique of inoculation into the gall-bladder has been well established by Henri Violle.[13]

The animal being fastened by its legs upon a metal table, the region of operation is shaved and sterilized with alcohol and tincture of iodin. The skin layers and the linea alba are incised; the peritoneum is divided along a grooved director. The liver, which is

[13] Thèse, Paris, 1912.

now in the field, is drawn down and turned from below upward and from behind forward, so that its posterior surface is brought into view. Its mass is next held wedged between gauze tampons underneath the dome of the diaphragm and in the space between the liver and intestines. A silk ligature on a *Reverdin* needle is then passed under the neck of the gall-bladder and the common bile duct, tied off.

The gall-bladder is entered at its free pole with a sterile 5 cc. syringe and the contents, often thick and viscous, are aspirated. By diluting this bile and washing the pocket several times with physiological salt solution until the latter flows back completely colorless, a sort of reservoir is formed with a capacity of 0.5 to 1 cc. and prepared to receive the bacillary suspension. The latter is drawn up into a second syringe which is attached to the original needle left always in place, and the injection gently made. A silk thread is next passed in such a way as to include in its loop both the needle and a small amount of surrounding gall-bladder wall which is held slightly retracted with forceps. As the needle is pulled out the thread is tightened so that no fluid can escape. Finally, to be still more sure, the orifice is cauterized. The tampons are removed and the linea alba closed with 3 or 4 catgut sutures. The skin layers are joined with a few skin hooks (agrafes de *Michel*). Finally the sutured wound is painted with tincture of iodin and covered with collodion.

The operation for direct injection into an intestinal loop is performed in the same way.

m. Intrapleural and intraarticular inoculations, etc.

The conditions of infection may be varied by employing other rather unusual modes of inoculation. At times it is desirable to introduce culture or tuberculous material directly into the pleural cavity or into a joint. The site of election for entering the pleural cavity either with needle alone or with needle and syringe is the 4th right intercostal space.

The joint cavities may be easily reached by forcibly extending the member.

C. CHOICE OF EXPERIMENTAL ANIMALS

Of all the animals ordinarily used in laboratories, the *guinea pig* and *monkey* are the most susceptible to tuberculous infection, par-

ticularly when one is using bacilli of the *human type*. The *rabbit* is more resistant but succumbs very quickly to inoculation with *bovine* bacilli, and with *avian* bacilli, especially if injected intravenously. One-tenth of a milligram of a culture of bovine bacilli (weighed in the fresh state) usually suffices to kill the rabbit in less than 2 months, while with human bacilli it is, as a rule, refractory to infection by the same path with even 10 mgm.

We shall soon have more to say upon the value of this difference of susceptibility on the part of the rabbit to human and bovine virus in the recognition of bacillus types (*Chapter XXI*).

The *dog* is infected only with difficulty; tuberculous infection can however be accomplished by ingestion or inoculation of sufficiently large doses of bacilli. The same applies to the *rat*.

The *mouse* is less susceptible.

A. Marmorek[14] found that this small rodent is more readily infected if a suspension of bacilli mixed with a small amount of solution of quinine hydrochlorate be injected into the peritoneum. This substance paralyzes the leucocytic defense for a certain period of time and permits the bacilli to remain longer in the general circulation, the result being that the lungs are the most extensively infected, although the liver and spleen are likewise full of tubercles.

Birds may serve for the study of *avian* tuberculosis. The *parrot*, the *goshawk* and the *parrakeet* are peculiar in that they are susceptible to both the mammalian and avian types of bacilli.

In chapters to follow we shall return to the important question of the respective capacities of different animal species to contract tuberculous infection, whether experimental or spontaneous.

[14] Berl. klin. Wchnschr., 1906, **43**, 328.

CHAPTER XXI

TUBERCLE BACILLI OF MAMMALS

Differential Characters of Human and Bovine Types

Almost all the higher vertebrates—mammals, birds, reptiles, batrachia and fish—are susceptible to infection by the tubercle bacillus; however, owing probably to the effects of long adaptation through the ages, we find now that this microörganism possesses special biological characteristics depending upon its origin in the tuberculous lesions of human or other mammals, birds or cold blooded animals. Gradually therefore races of tubercle bacilli have been developed whose specificity is more or less sharply defined in relation to this or that animal species; and of each of these types we should make a separate study.

The bacillus of mammals, which will first be considered, does not escape this law of adaptation. The works of Theobald Smith[1] (1896–1898), of Frothingham (1897), of Dinwiddie[2] (1899) and then those of Robert Koch and Schuetz[3] (1902), to which we shall return, brought out the fact that the bacillus commonly isolated from chronic tuberculous lesions in man is *ordinarily* avirulent for cattle. On the other hand it seems fairly certain that the bacillus of bovine origin is relatively avirulent for man. Moreover these bacilli possess certain special biological characteristics which often enable one to establish their source, whether human or bovine, but their specificity is not absolute. They belong without any question to the same race (*Bacillus tuberculosis mammalium*) and generate lesions of like nature in the invaded organism. The same artificial media serve to grow them and external agents, physical or chemical, have similar effects upon them. They will be distinguished therefore simply as *human type* and *bovine type*.

[1] J. Exper. Med., 1898, **3**, 451.
[2] Arkansas Agric. Exper. Sta. Bull. No. 57, 1899.
[3] Arch. f. Tierheilk., 1902, **28**, 169.

A. DIFFERENTIAL CHARACTERS OF HUMAN AND BOVINE TYPES AS REGARDS MORPHOLOGY AND CULTURE

Some observers state that bovine bacilli on glycerinated gelatin serum are rather thick and short; their length scarcely exceeds 1 micron, while human bacilli are longer (about 3 microns) and at the same time are slender, often curved and more fragmented after staining with Ziehl (Th. Smith, Ravenel).[4] These differences however are neither very marked nor constant.

In 1903, Th. Smith[5] observed that the acidity curves of glycerin broths in which human and bovine bacilli respectively are being cultivated, differ when repeatedly tested during a period of 3 to 4 months. In the case of the human type the acidity increases rapidly from the beginning up to the fifteenth or eighteenth day. It then falls until about the thirty-second day and from then on remains stationary without the broth ever becoming definitely alkaline.

The bovine type diminishes the acidity of the medium or even renders it weakly alkaline, and the curve tends slowly downward.

To test this point, one uses a 100 cc. Erlenmeyer flask into which glycerinated broth is poured to a depth of 1.5 cm. The cotton plug should be covered with rubber or tinfoil to avoid evaporation in the incubator. The broth at the beginning of the experiment should contain 5 per cent of glycerin, so that some at least will remain after the bacillus shall have used up a portion. Its acidity will be from 1.8 per cent to 2.2 per cent (expressed in terms of 1/20 normal HCl).

Th. Smith believes that the difference between the human and bovine types lies in the fact that the latter utilizes glycerin without decomposing it into acids, whereas the human bacillus converts it into acids.

According to M. Grund,[6] this reaction is not absolutely constant inasmuch as intermediary types are found and, at times even, types which behave in the reverse manner. This is also the opinion of G. Wankel[7] from studies which he made at the Robert Koch Institute on 45 cultures of known origin.

The respective cultural characteristics on fluid or solid media give

[4] Proc. Path. Soc. of Philadelphia, 1900, iii, 1902, v.
[5] J. Med. Research, 1904, **13**, 253; 405; 1910, **23**, 185.
[6] Ibid., 1911, **25**, 335.
[7] Deutsch. med. Wchnschr., 1913, **39**, 2461.

indications which one should not fail to utilize. Kossel, Weber and Heuss, afterward Oehlecker,[8] recommend the use of 4 per cent glycerin broth as a differential medium. The human type grows vigorously upon it, covers the surface of the liquid in 2 or 3 weeks, reaches upward on the sides of the flask, and forms a thick wrinkled floating membrane. The bovine type grows on it only slowly and with difficulty, in the form of a pellicle or an extremely thin film. These differences however hold good only for strains of bacilli recently isolated on solid media (glycerinated gelatin serum), and L. Rabinowitsch,[9] J. Fibiger, and Jensen[10] find them inconstant.

Moeller, and then Beck, had noticed that the addition of glycerin to serum or Dorset's egg medium hastens the growth of human type bacilli, while definitely retarding that of the bovine type.

Park[11] made use of this observation after having verified its correctness, and from many trials was able to state that the best medium for isolating and differentiating the bovine bacillus is that of Dorset (non-glycerinated egg supplemented with water to the extent of 10 per cent of the volume, then coagulated), and for the *human* bacillus that of Lubenau (egg to which is added 30 per cent of its weight of alkaline broth glycerinated to 5 per cent, and then coagulated). If a few tubes of each medium are inoculated with the tuberculous material in question, it is found that the glycerinated medium is constantly unfavorable to the growth of the *bovine* type, whereas the *human* type almost always grows readily from the beginning. On the other hand, on non-glycerinated egg, the bovine bacilli form very small, soft, moist, glistening colonies which should be spread over the medium soon after becoming visible. They thus acquire more vigor and may afterward be transplanted to glycerinated potato where they grow well.

Park's opinion is that all cultures which grow abundantly on glycerinated egg at the first sowing are of the human type. All others which, to the contrary, do not grow on glycerinated egg, but do grow on non-glycerinated egg, are of the *bovine* type.

On the egg broth of Besredka[12] (*see Chapter II, C*), the human

[8] Tuberk.-Arb. a. d. k. Gsndhtsamte, 1905, H. 1/3; 1907, H. 7, 65.

[9] Berl. klin. Wchnschr., 1906, **43**, 784.

[10] Ibid., 1904, **41**, 129; 171; 1907, **44**, 93; 134; 1908, **45**, 1876; 1926; 1977.

[11] Studies Research Lab., Dept. of Health, N. Y. City, 1908, 1909, 1910.

[12] Ann. de l'Inst. Pasteur, 1913, **27**, 1009.

bacillus after 4 to 6 weeks produces small, more or less dry, non-adherent scales, while the bovine bacillus forms a layer of filaments of muco-membranous appearance which spreads over the bottom of the flask.

In the course of certain investigations carried out by C. Guérin[13] and myself on the culture of tubercle bacilli on potato cooked in 4 per cent glycerin ox bile, we called attention to the fact that bacilli of *human* type develop only with great difficulty and very sluggishly in the presence of *ox bile* whereas they develop readily in the presence of *human bile.* Inversely the bovine bacillus grows quickly and abundantly on potato with ox bile, while culture is very difficult on media with human bile. This fact enabled us to establish the *bovine* origin of a bacillus isolated by Salimbeni, at the Pasteur Hospital, from the mesenteric glands of an infant who died of acute miliary tuberculosis at the age of 5 months.

B. DIFFERENTIAL CHARACTERISTICS OF HUMAN AND BOVINE TYPES IN EXPERIMENTAL INOCULATION

All investigators today are agreed that the best procedure for differentiation consists in inoculating cultures of the first or second generation into the rabbit. As early as 1868 Villemin had remarked that sputum of phthisical patients was only slightly virulent for this animal, while tuberculous matter from cattle was much more so. Later on, Orth, Baumgarten, then Theobald Smith in 1896 and 1898, Vagedes[14] in 1898, made similar observations and Kossel, Weber and Heuss[15] showed that there is an almost complete parallelism in these differences of virulence for the rabbit and for the calf. These differences are particularly marked when small doses are injected *intravenously.*

Park and Krumwiede[16] have adopted an excellent technique. They inoculate four 1500 to 2000 gm. rabbits with a 3 to 4 weeks old growth of the second or third generation of the culture in question. The latter should be taken from glycerin or non-glycerin egg or from glycerin potato medium. Two of the rabbits receive 1 mgm., while

[13] Compt. rend. Acad. des sci., 1909, **149**, 191.
[14] Ztschr. f. Hyg., 1898, **28**, 276.
[15] Tuberk.-Arb. a. d. k. Gsndhtsamte, 1905, H. 3, 30.
[16] J. Med. Research, 1910, **23**, 205; 1911, **25**, 313.

the other two receive 0.01 mgm. of a fine suspension in the marginal ear vein. Each animal is weighed regularly. Those not dying are killed after 60 days.

By this method it is found that the rabbits receiving *human* bacilli, even 1 mgm., never die during this period and autopsy reveals only non-progressive discrete lesions of the lungs or kidneys; at times even no lesions at all. *Bovine* bacilli, on the contrary, most frequently produce a generalized tuberculosis, even with 0.01 mgm., while the dose of 1 mgm. often leads to a rapidly fatal intoxication. If the rabbits die within 30 to 60 days, with or without generalized lesions, it may be concluded that the type is *bovine.*

Among 66 rabbits inoculated by Park with 0.01 mgm. of *bovine* bacilli isolated from human tuberculous lesions, only 9 had to be sacrificed on the sixtieth day. All the others had succumbed, for the most part between the thirtieth and fiftieth day. On the other hand, among 427 rabbits inoculated with 1 mgm. of *human* bacilli 235 were killed after 60 days and of the latter 175 had increased in weight.

According to F. Schick and F. Krusius[17] the human type may be quickly differentiated from the bovine, from the diagnostic point of view, by inoculating a sufficiently dilute suspension of pure culture *into the anterior eye chamber of the rabbit.* The human type by this method produces an attenuated tuberculosis, non-progressive or curable, of the cornea and iris, while the bovine type quickly destroys the eye and gives rise to a grave infection which rapidly becomes generalized. The optimum dose of virus is one millionth of a milligram, which is about 40 bacilli.

This differentiation may also be carried out by intravenous inoculation into one of the lateral tail veins of the white mouse, as Trommsdorff[18] has proposed. W. Binder[19] found thus that these small animals died within 27 to 57 days after the inoculation of 1 mgm. of bovine bacilli, while the human bacillus, in the same dose, did not cause death until after 130 to 188 days, and only with very discrete lesions.

Aoki[20] also proposed to make use of the rat. He infected 81 of them, some intraperitoneally, others intravenously. Among this

[17] Veröffentl. d. R. Koch Stift. z. Bekämpf. d. Tuberk., 1913, H. 5/6, 133.
[18] Arb. a. d. k. Gsndhtsamte, 1909, **32**, 568.
[19] Bericht. über d. Veterinarinst. Leipz., 1913.
[20] Ztschr. f. Hyg., 1913, **75**, 62.

number there were 35 which received 6 different strains of *bovine* type and 46 which received 8 different strains of *human* type. Nine animals of the first lot became tuberculous, while 26 showed nothing abnormal. On the contrary, in the second lot (*human* type) 42 of 46 became tuberculous. It seems therefore that *human bacilli are more virulent for the rat* than are *bovine* bacilli.

Fraser[21] is of the opinion that a differentiation can be made with assurance by inoculating the synovial membrane of the joints of the rabbit's paws.

If the bacillus is of human type, the membrane becomes thickened and, apart from a little fluid secretion within the serosa, the animal suffers no inconvenience. Three or four months later it is found that the synovial membrane is the seat of a chronic tuberculosis. Only rarely is the infection disseminated.

If bovine bacilli have been injected, the animal, some ten days afterward develops a sharp pain and holds up its feet, then begins to grow thin. Three or four weeks later the joint cavity becomes filled with caseous pus; the cartilages become eroded and tuberculous foci form in the bones and viscera.

Either cultures or pathological material may be spread upon the freshly shaved or epilated skin of the guinea pig, as was done by E. Tomarkin and S. Peschic.[22] With the human bacillus, infection is sluggish and inconstant, while with the bovine type it is said always to occur and to assume a much more active form.

I have demonstrated, with C. Guérin, that the lactating goat is likewise an excellent means of differentiation. By means of a fine milking tube and a sterilizable syringe, with care not to wound the mammary gland, a suspension containing 1 to 2 mgm. of culture is introduced deeply into one of the two teats. If the bacilli are of *human* origin, a more or less intense local infection results, persisting several months and then disappearing to reappear in the same teat during the period of lactation following a new pregnancy. Infection however remains local and does not become generalized. If, on the other hand, bacilli of the *bovine* type are injected under the same conditions, the tuberculous infection, at first circumscribed, quickly spreads by way of the lymphatics. Very soon it invades the pelvic glands, the lungs, and the tracheo-bronchial and mediastinal glands. Finally the animal dies in 4 to 5 months.

[21] Brit. M. J.,1912, ii, 1432.

[22] Deutsch. med. Wchnschr., 1912, 38, 1032.

Virulence for cattle is best tested by the method of Kossel, Weber and Heuss,[23] by inoculating subcutaneously behind the shoulder 50 mgm. of bacilli weighed in the fresh state and suspended in 5 cc. of physiological salt solution. Under these conditions *human* type virus causes the formation of rather large local abscesses, but is incapable of causing a generalized tuberculosis, while most strains of the *bovine* type bring about rapidly progressive and uniformly serious lesions. Park tested in this manner 8 cultures of *bovine* type from *human* sources. Six caused generalized tuberculosis fatal in 23 to 63 days; one gave rise to an extensive but retrogressive tuberculosis, the animal remaining in a state of apparently good health; one showed itself to be avirulent and the control rabbit proved that the original virulence of that strain had disappeared.

It would seem therefore that the parallelism in virulence for the rabbit and calf is indeed a real one, as the experiments made at Berlin by a Commission of the *Kaiserliches Gesundheitsamt* had already indicated.

However it must be recognized that certain cultures show atypical characteristics as regards virulence and that it may be extremely difficult or impossible to establish their type (O. Malm).[24] Here the opinion is justified that one is dealing with types which are poorly adapted to the bovine or human body, or perhaps in some cases with a mixed infection, the possibility of which had been proved by L. Rabinowitsch[25] and W. Park.

One of the most curious examples in this respect is that of the so-called Schroeder and Mietzsch culture isolated at the sanatorium of Schömberg from the sputum of a tuberculous woman 29 years old. This culture, which was studied with great care by several experimenters particularly by Dieterlen at the *KK. Gesundheitsamt* and then by Ernst A. Lindemann,[26] showed itself constantly virulent for the rabbit and avirulent for the calf. It was likewise avirulent for the hen, which excluded an avian origin. The bacillus could therefore only be one of human type with atypical virulence.

In all cases one must be guarded in asserting too positively the human or bovine nature of a bacillus before testing it by all the vari-

[23] Tuberk.-Arb. a. d. k. Gsndhtsamte, 1905, H. 1, 1.
[24] Centralbl. f. Bakt., 1912, **65**, 42.
[25] Internat. Congr. on Tuberculosis, 10th, Rome, 1912.
[26] Arb. a. d. k. Gsndhtsamte, 1913, **45**, 197.

ous means today available. Experimentation must, as far as possible, be carried out with *pure cultures isolated from separate colonies.* The cultures should be inoculated into several rabbits; into some a uniform dose of 0.01 mgm. intravenously; into others a dose of 10 mgm. subcutaneously behind the shoulder or into the internal surface of the thigh. At the same time, in order to eliminate the possibility of avian origin, several hens should be inoculated intravenously with doses of 20, 5, 1, 0.1 and 0.01 mgm. respectively. Animals not dying within about six months should be autopsied at the end of that period. Careful examination of their lesions will give valuable information, but it must be understood that, as a matter of practical application, diagnosis can be established positively only for the bovine bacillus, either by mammary infection of the lactating goat or by the inoculation of 50 mgm. of culture under the skin of a calf.

C. VIRULENCE OF HUMAN TYPE BACILLI FOR CATTLE.—ATTEMPTS TO TRANSFORM THE HUMAN TYPE INTO THE BOVINE TYPE

As early as 1868 Chauveau had succeeded in demonstrating that if young cattle ingest infectious material from phthisical cases (products obtained by grinding pulmonary tubercles, contents of cold abscesses, or sputa) or if this material is injected either intravenously or subcutaneously, more or less extensive lesions develop in the glands, lungs and various viscera.

The transmissibility of human tuberculosis to cattle was therefore manifest and many other investigators have since accepted this fact. Thus Bollinger,[27] on introducing an emulsion from the lung of a phthisical patient into the peritoneal cavity of a calf, found, when he killed the animal 7 months later, that the mesentery and peritoneum were covered with fungoid masses which were altogether similar to those which characterize tuberculosis of cattle (pommelière).

Klebs,[28] Crookshank,[29] Sydney-Martin,[30] Thomassen,[31] S. Arloing,[32]

[27] München. med. Wchnschr., 1894, **41**, 85.
[28] Virchow's Arch., 1870, **49**, 292.
[29] Trans. Path. Soc., Lond., 1891.
[30] Rep. Roy. Comm. on Tuberc., 1895.
[31] J. Comp. Path. & Therap., 1901, p. 259.
[32] Bull. Acad. méd., 1901, **46**, 897.

Nocard,[33] De Jongh,[34] Ravenel,[35] and others, performed many equally conclusive experiments to the same effect.

The identity of human and bovine tuberculous virus seemed therefore firmly established when Robert Koch's communication at the 1901 Congress in London made it necessary to reexamine the previously obtained results and, at the same time, conduct further research.

It had to be admitted from the first that, it order to produce fatal tuberculosis in cattle, it was necessary to inject human bacilli or virulent human material in large quantity; to introduce them directly into the blood stream or peritoneum, or again, as Nocard had done, under the dura mater of the brain. Infection by the digestive tract was as a rule a matter of repeated ingestions of infectious material if lesions of a fatal nature were to be produced, and even then one was not always successful.

It was settled at last that the virulence of human tuberculous products for cattle was very inconstant and generally quite low, so that the statements of R. Koch and Schuetz, who had carried out the majority of their inoculation experiments with phthisical sputa, were verified in large part.

It cannot be questioned that bacilli isolated from the sputum of phthisical patients are almost *constantly* of *human* type. Of 632 cultures thus isolated in different countries of the world, Mollers[36] finds only one which can be assigned to the bovine group and that is from a doubtful case (de Jong-Steuermann).

But if, instead of employing sputum or pure cultures derived from guinea pigs inoculated with sputum, one uses, in order to infect the cattle, ground up pieces of tuberculous organs other than the lung, particularly glands, or cultures from guinea pigs inoculated with these organs, a severe infection usually results which progresses rapidly and finally becomes generalized. In such a case, Kossel, Weber and Heuss grant that one is dealing with human infection of bovine type, and that in reality the cattle have been inoculated with bovine bacilli.

This interpretation is open to question.

[33] Rev. Vét., 1902, Jan., 49.

[34] Semaine méd., 1902, **22**, 17.

[35] Proc. Path. Soc., Philadelphia, 1902, May.

[36] Deutsch. med. Wchnschr., 1911, **37**, 341.

What is not open to question, on account of the mass of experimental evidence, is that sputa from phthisical patients or pure cultures isolated from them are,—except with very rare exceptions,—incapable of immediately producing in cattle an active tuberculosis which goes on to miliary disease or to the chronic fatal forms. These bacilli, inoculated subcutaneously, produce lesions which as a general rule remain localized. When introduced into the udder of the goat or cow they cause an engorgement of the supra- or retro-mammary glands, without tendency to extension. When injected into the blood stream in large doses, they cause disseminated lesions, ganglio-pulmonary or nodular lesions in the peritoneum, which likewise are not extensive. In small doses they are tolerated perfectly and we shall see later (*Chapter XLII*) that they even serve to vaccinate. Finally, when inhaled, they create peri-alveolar lesions which remain very limited or may even be entirely harmless (R. Koch and Schütz, Moeller, Kossel, Weber and Heuss).

Nevertheless many experiments have served to demonstrate that tuberculous material from man, or bacilli isolated from this material and possessing all the morphological and cultural characters commonly attributed to the human type, manifest at times a virulence for cattle which apparently equals that of the most authentic bovine types.

Orth[37] in collaboration with Esser, Westenhoeffer,[38] J. Fibiger and Jensen,[39] Dammann and Müssemeier,[40] Eber[41] at the Veterinary Institute of Leipzig, Delépine, Hamilton and Young, Schottelius, the English Royal Commission in its final report of 1911 and many other observers have furnished examples of this.

In all of these cases indeed there is no certainty whatever that one is not dealing with bacilli whose bovine origin is relatively close. It may always be argued that these *human* strains, virulent for cattle, had original characters which were poorly defined or unstable and that this is the reason why the characters are still uncertain and why between the *human* and *bovine* types, as ordinarily defined, there exists a whole series of intermediaries which approach more or less either the one or the other.

[37] Deutsch. med. Wchnschr., 1903, **29**, Ver.-Beil., 244.
[38] Ibid., 1903, **29**, 221.
[39] Ibid., 1904, **30**, 321; 402.
[40] Centralbl. f. Bakt., Ref., 1906, **38**, 336.
[41] Ibid., Orig., 1911, **59**, 193; 1913, **70**, 229.

It follows from this that we are not justified in inferring the *duality of human and bovine tuberculous virus*, as Robert Koch[42] would have it. These types differ one from another only because they have more or less adapted themselves, through a series of successive cultural generations to a *human* or *bovine* environment. And it cannot be conceived that cultures of *human* or *bovine* bacilli, attenuated by prolonged existence on artificial laboratory media and become more or less avirulent, constitute special types. In all of these cases we have to do with the same bacillus.

Many efforts have been made in a variety of ways to convert what by agreement is called the *human* type into the *bovine* type, for example by inoculating successively the same virus from man to cattle, then from cattle to cattle or through an intermediate stage in the goat. It would seem that in a few cases partial success has been attained. The facts published by Eber are particularly interesting in this respect.

Von Behring,[43] Römer and Ruppel found that a human bacillus isolated from the sputum of a phthisical patient had become virulent for cattle after six months in the body of a goat. Cultures obtained from it, after three passages through the guinea pig, exhibited the morphological appearance of the bovine type. Römer admits that such changes are exceptional. Dammann and Müssemeier,[44] and later De Jongh,[45] claim that they have obtained the same results through the intermedium of the goat.

The Royal English Commission[46] reports upon a culture isolated from the knee joint of a patient of 38 years and which was inoculated into a calf. The resulting lesions were re-inoculated into a second calf. The two animals developed a local tuberculosis with extension to the regional glands, but only after 67 to 81 days. When carried over to a third calf, this virus produced more extensive lesions, with numerous tuberculous nodules in the lungs and a few in the spleen and liver. Emulsions of the prescapular glands from this calf pro-

[42] Internat. Congr. on Tuberculosis, 6th Wash., 1908.

[43] Behringswerke Mitt., 1907.

[44] *Untersuchungen über die Beziehungen zwischen der Tuberkulose des Menschen und der Tiere.* Hanover, 1905, Schaper.

[45] Internat. Congr. on Veterin. Med., 8th, Budapest, 1905.

[46] Great Brit. Roy. Comm. on Tuberculosis, Rep., Lond., 1904, 1911, Wyman & Sons.

duced a sluggish but generalized and fatal tuberculosis in a fourth calf, and an equally generalized and fatal tuberculosis in a rabbit.

Cultures isolated from the first and third calves, and from guinea pigs which had received the original virus, showed the human type. After the fourth calf, the cultures assumed the characteristics of the bovine type.

Using the spleen of guinea pigs inoculated with human pulmonary lesions, Eber[47] (of Leipzig) successfully infected calves by intraperitoneal injection. From the lesions in the calves (nodules in the peritoneal glands) he isolated bacilli which in cultures and in cattle behaved like true bovine bacilli, whereas the cultures derived directly from the human tuberculous lesions had the characters of the human type of bacillus.

These various positive results as well as others reported by Malm, Park and Krumwiede, and others, have been the subject of a lively controversy on the part of Neufeld, Dold and Lindemann[48] on the ground that the experiments as performed do not exclude possible sources of error through spontaneous bovine infection. The question therefore of experimental transformation of the human type of bacillus into the bovine type cannot yet be regarded as settled.

Further on we shall see that there has been no greater success in artificially transforming the bovine type into the human type, and yet no one today denies that bovine bacilli may infect man and particularly the child, inasmuch as 6 to 10 per cent of cases of fatal tuberculosis in early life, that is under 5 years, are to be attributed to the bovine bacillus, according to the statistics of Park.

So it must be granted that man and cattle may be infected reciprocally, but that the characters acquired through the adaptation of the tubercle bacillus to man (and which cause it to be called of human type) render it much less virulent for cattle than is the bacillus of bovine origin.

Inversely, we shall see in another chapter (XXV) that tuberculous cattle may infect man, but that they are above all a danger to their own kind or, to be more exact, to their own kind stabled with them.

The fact that human tuberculosis is very widespread and fatal in countries where bovine tuberculosis either does not exist or is

[47] München. med. Wchnschr., 1910, **57**, 115:—Centralbl. f. Bakt., 1911, **59**, 193; 1913, **70**, 229.

[48] Centralbl. f. Bakt., 1912, **65**, 467.

extremely rare, is evidence moreover that man's tuberculosis is propagated throughout the world by man himself.

In India, Indo-China, Japan, the Philippines, Oceania, in the whole of North and West Africa, in the majority of the countries of South America, in the arctic regions such as Alaska, Greenland, Lapland, and even in certain countries of Europe, for instance in England in Herefordshire or in the islands of Jersey and Guernsey, in Sicily, in Tuscany, in Sardinia according to Gosio,[49] cattle are practically free from tuberculous infection and yet the human death rate from tuberculosis is very considerable (*see Chapter XL*).

In these various countries interhuman contagion is obviously the only factor entering into the infection of man.

[49] Bull. de l'Office internat. d'hyg. publique, 1912, **4**, 1380.

CHAPTER XXII

BOVINE TUBERCULOSIS

Its Pathologico-Anatomical Characters

Tuberculous infection of cattle generally manifests itself by slowly progressive lesions. Acute forms are only exceptionally observed unless induced by massive experimental inoculations.

Exclusively glandular localizations are the most common, with the pulmonary next in frequency. In order to follow their evolution it is well to have a clear idea of the anatomy of the lymphatic system, of which a sufficiently accurate diagram is given in figures 20, 21 and 22. Then come, in order of frequency, localizations in the serous membranes (pleural or peritoneal), intestinal tuberculosis, tuberculosis of the udder and generalized infections.

In the great majority of cases the symptoms of the disease pass unnoticed for a long time and the tuberculous animal appears to be in perfectly good health. Only after the involvement of some important organ does clinical examination permit of establishing the diagnosis, but even then it can be made definitely only when substantiated by such evidence as the tuberculin test or discovery of the bacillus.

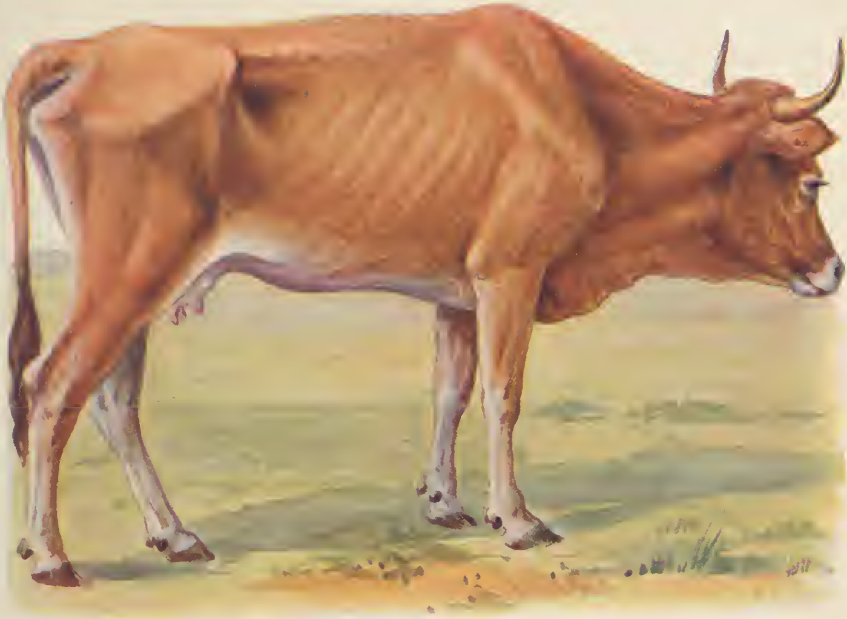
Young animals grow irregularly and slowly, and appear delicate and sickly.

Adult animals seriously affected are usually emaciated; the flanks are sunken, the hairy coat is lustreless and rough and the hide dry and adherent to the subjacent muscles (*see Plate XIII*). The dull blear eyes are deep in their sockets, the expression is one of dejection, and the head is held extended. Masses of flesh atrophy and bony prominences become exaggerated. Often there are meteorism and diarrhoea.

In time the animals become cachectic. Their temperature, at first normal, afterward irregular, mounts little by little with fluctuations up to 41°C. in the evening. Respirations become short, rapid and labored, while coughing is frequent and accompanied by a yellowish fetid discharge. Pinching of the back bone seems to cause

PLATE XIII

1. Tuberculous cow from the region of Savoy (tarentaise).
2. Vegetative tuberculosis of the pleura in the cow (Abattoir at Lille).



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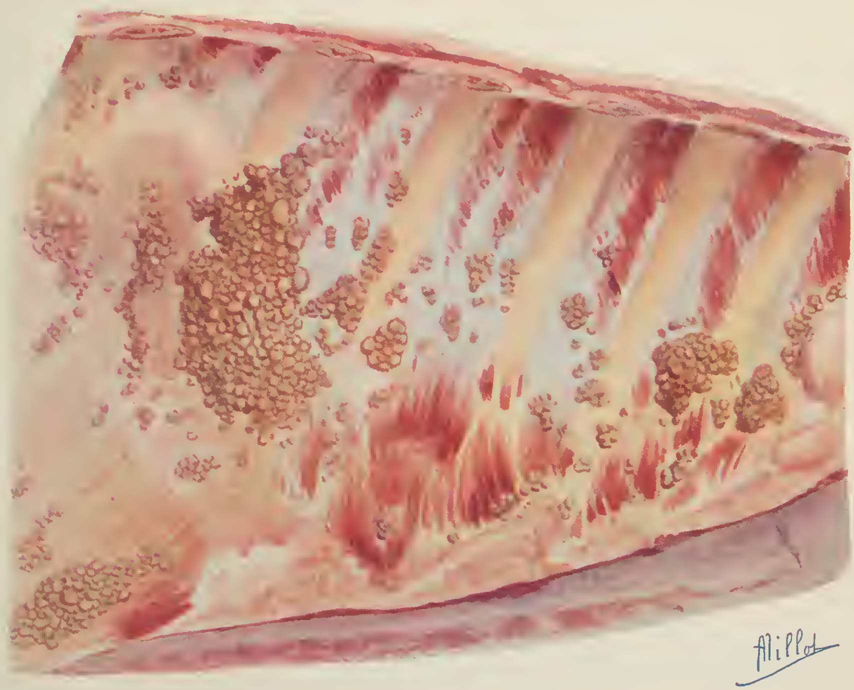

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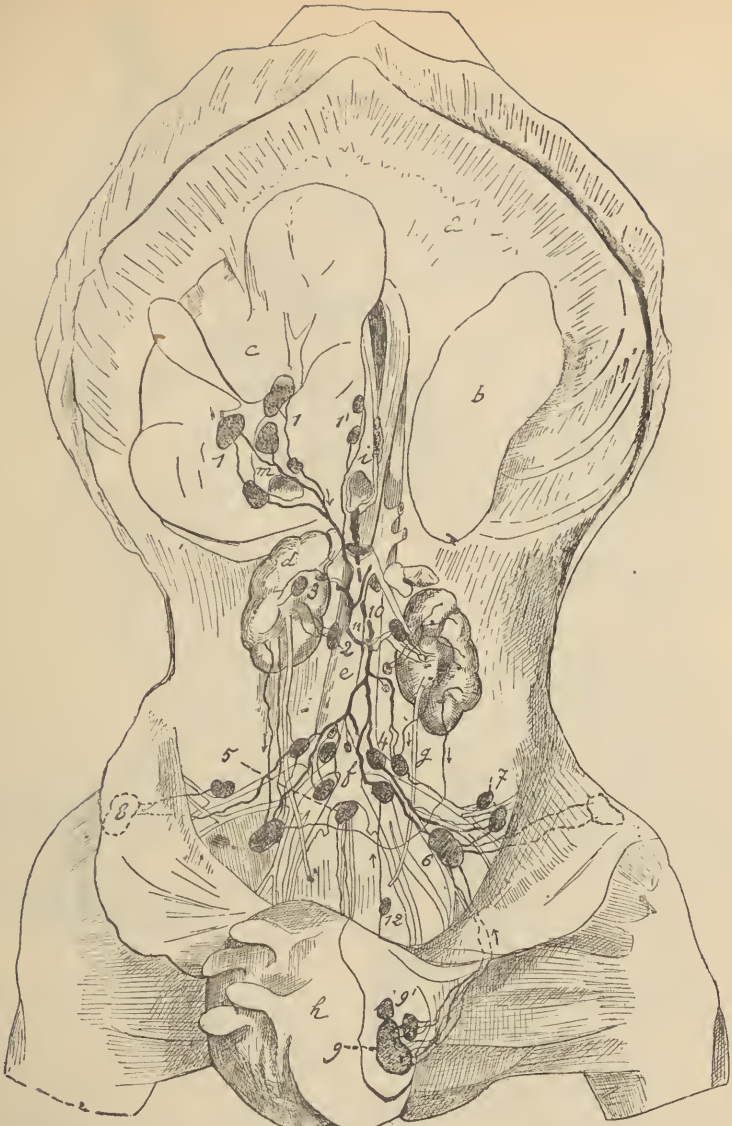


FIG. 20. SCHEMA OF THE ABDOMINAL LYMPHATIC GLAND SYSTEM IN CATTLE (DRAWN BY L. BRUYANT, AFTER HERMANN BAUM*)

a, Diaphragm; *b*, spleen; *c*, liver; *d*, kidney; *e*, vena cava; *f*, aorta; *g*, ureter; *h*, mammary gland.

1. Hepatic glands
1'. Accessory hepatic glands
2. Aortic glands
3. Renal glands
4. Median iliac glands
5. Hypogastric glands
6. Deep inguinal glands
7. Lateral iliac glands
8. Sub-iliac glands
9 and 9'. Superficial inguinal glands
10. Dorsal lymphatic channel
11. Intestinal trunk
12. Internal sacral gland

* *Das Lymphgefässystem des Rindes;* Berlin, 1912.

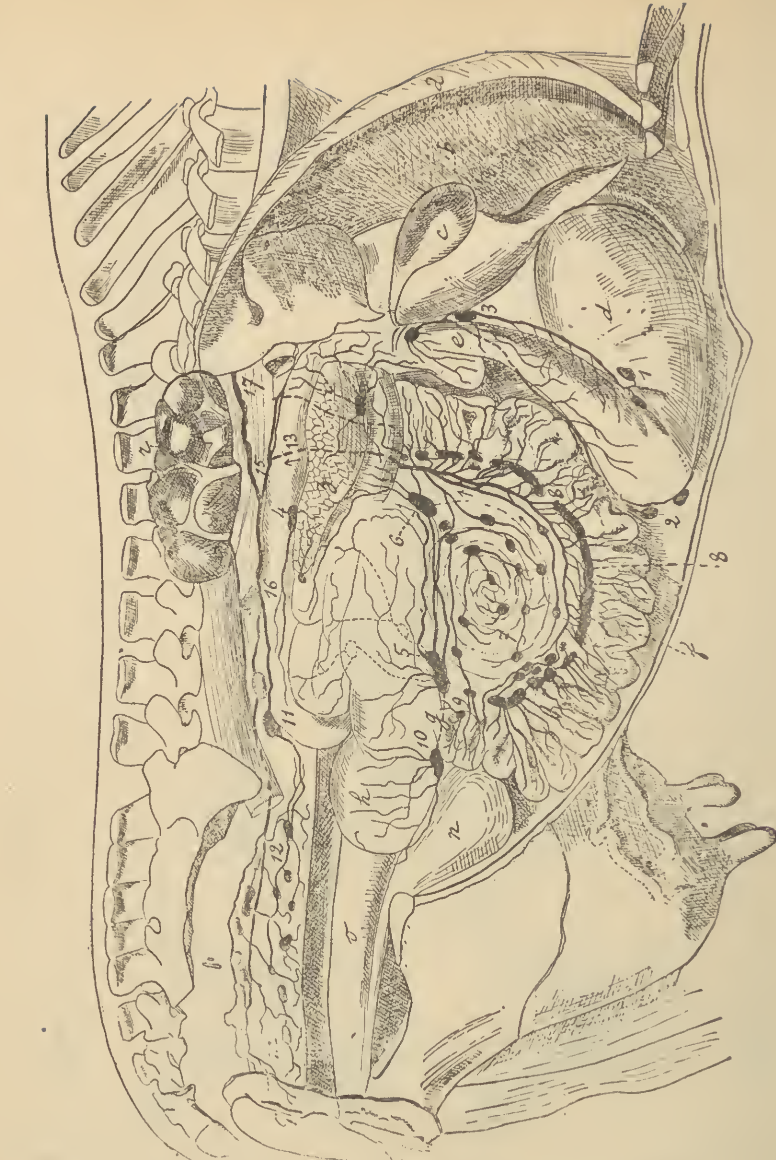


Fig. 21. Schema of the Intestinal Lymphatic Gland System in Cattle (Drawn by L. Bruyant, after Hermann Baum)

a, Diaphragm; *b*, liver; *c*, gall-bladder; *d*, the first stomach; *e*, duodenum; *f*, jejunum; *g*, ileum; *h*, caecum; *k*, mesentery; *m*, rectum; *n*, bladder; *o*, vagina; *p*, vulva; *q*, anus; *r*, right kidney (drawn back); *z*, pancreas.

1 and 2'. Dorsal and abdominal lymphatic glands of stomach
3. Hepatic glands *4*. Pancreatic-abdominal glands
5, 6, 7, 8 and 9. Glands of the small intestine
10. Glands of the caecum *11*. Median iliac glands *12*. Ano-rectal glands
13. Common trunk of the intestinal lymphatic glands
14. Common trunk of the gastric lymphatic glands
15. Intestinal trunk of glands *16*. The lumbar lymphatic canal
17. Receptaculum chyli

pain. The appetite disappears, and rumination becomes irregular and slow. At last death intervenes, either through general weakness or as the result of some fatal extension of the disease.

According as these localizations are more or less limited or extensive several types of bovine tuberculosis are distinguished, on the basis chiefly of their pathological anatomy. Ostertag[1] gives the relative

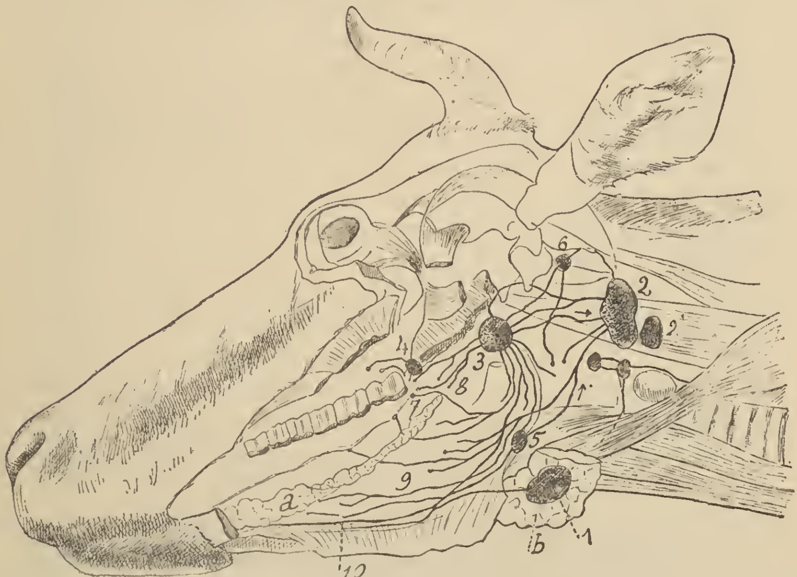


FIG. 22. SCHEMA OF THE LYMPHATIC GLAND SYSTEM OF THE TONGUE AND MAXILLARY REGION IN CATTLE (DRAWN BY L. BRUYANT, AFTER HERMANN BAUM)

1. Maxillary gland
2 and 2'. Retro-pharyngeal lateral gland
3. Retro-pharyngeal median gland
4. Pterygoid gland
5. Hyoid oral gland
6. Hyoid aboral gland
7 and 8. Lymphatics of the superior maxillary
9. Lymphatics of the base of the tongue
10. Lymphatics of the tip of the tongue. *a*, sublingual gland; *b*, submaxillary gland

[1] Internat. Congr. of Hyg. and Dem., 13th, Brussels, 1903.

frequency of the different types, based on more than 43,000 observations in the statistics of the German abattoirs, as follows:[2]

	per cent
Tuberculosis generalized	10.7
Tuberculosis localized to a single organ	50.0
Tuberculosis localized to one cavity	17.0
Tuberculosis localized to several cavities	19.5

And for the different organs involved the following table of percentages is given:

	per cent
Lungs	75.0
Visceral pleura	55.0
Peritoneum	48.0
Costal pleura	7.0
Liver	28.0
Spleen	19.0
Trachea	3.0
Intestine	1.0
Heart	0.9
Kidneys	0.7
Bones	0.4
Diaphragm	0.2
Larynx	0.13
Brain	0.04
Spinal cord	0.03
Tongue	0.01

Pulmonary localizations in cattle, instead of leading to the formation of multiple cavities as in man, are characterized by the formation of cavities communicating directly with the exterior by means of the bronchi, and more or less surrounded by thick partitions of dense connective tissue. This difference in the lesions is due to differences in anatomy. In cattle the lung lobules are limited by a loose, very abundant elastic tissue which has a great tendency to become infiltrated, so that each lobule may be separated from its neighbor by a layer of serous fluid two or three millimeters thick.

The tuberculous foci develop as do the different processes already described in connection with the histogenesis of the tubercle (*Chapter VI*). They may become encysted within a sclerotic enveloping capsule, or break down to form purulent abscesses which empty their contents either into the bronchi, or into the lymphatic spaces, or into the neighboring blood vessels. The result is then at times an apparent cure or it may cause a more or less abundant and repeated reinfection which finally spreads the virus to the neighboring or distant tissues or organs

[2] *Maladies microbiennes des animaux,* 3rd edit., Paris, 1903, Masson & Cie.

A. PULMONARY LOCALIZATIONS

We can do no better than borrow from Nocard and Leclainche their very excellent description of tuberculous lesions in cattle, the appearance of which, according to them, varies with age, extent, and mode of evolution:

"The initial changes consist in groups of tuberculous follicles forming small grayish masses, isolated or coalescent, collected into lobular foci or into irregular strands. The isolated miliary tubercle appears as a little rounded mass the size of a millet seed, grayish, translucent, homogeneous, surrounded by a slightly inflammatory areola (*granulation grise* of Laennec). The appearance is very soon modified by degeneration of the central portions; the size of the focus increases and an opaque yellowish white spot is to be made out at the center. In a third phase, the peripheral portions, now thickened and compressed, make up a resistant fibrous shell. On section the contents are found caseous, yellowish in color, pasty in consistency, and containing calcareous granules which are detected by crushing between the fingers (*tubercule cru* of Laennec).

"Adjacent tubercles fuse together to make up rounded nodules, of varying dimensions, isolated and distributed irregularly in the mass of the two lobes, or confluent and localized in one or several foci.

"New tuberculous eruptions occur in the parts which have remained unaffected and changes of all ages are to be found superimposed in a single area. Functioning tissue becomes more and more reduced and the lesions acquire at times a considerable size. Certain foci undergo purulent liquefaction. Abscesses, limited by thick fibrous capsules, contain a grumous pus which is yellow or greenish in color and of variable consistency. Such an abscess may remain confined for a long time or, indeed, indefinitely; the enveloping capsule becomes more compact; the lesion retains its same characteristics, or may slowly undergo cicatrization with resorption of its contents and transformation into sclerotic tissue. Often moreover abscesses ulcerate through their walls; they empty their contents into a bronchus and form small or large cavities.

"When there are old and extensive ulcerations the lung becomes only partly collapsed; its weight reaches 20 to 30 kgms. and even more; its surface is covered with bosses; the pleura is thickened where it overlies the tumors and is covered with fibrinous plaques which

are dry and adherent or with fibrous neoplasms or tuberculous vegetations (*pommelière*). The bosses are rounded, confluent and of greatly varying size. Certain of them are hard and resistant and grit under the knife, on section their tissue is yellow and rough to the touch. The softened foci contain a caseous substance with calcareous granules. Others are more or less fluctuating, and section discloses a thick, yellow, grumous material, like mortar. Some foci contain yellow, thick viscous pus. Section of the lung shows cavities with the lining surface sprinkled with poorly developed granulations, pale in color and covered over with fetid pus. Certain cavities, extensive and convoluted, are traversed by strands made up of large bronchi or vessels which have resisted purulent softening (*Plate XIII*).

"Most often the areas of tuberculosis are surrounded by healthy pulmonary tissue which has preserved its pink color, its flexibility, elasticity and normal texture; in some cases there exists a peripheral zone of hepatization. In others the foci are set apart in the center of a mass of sclerotic tissue, fibrous, white, and very resistant.

"In some diseased cattle, the lung is full of rounded nodules, from the size of a hazel-nut to that of a chestnut, of a dirty white color, firm and homogeneous in consistency throughout, and with no central softening. They are the *fibrous tubercles* of the early authors, more often observed in the horse.

"Foci of caseous pneumonia are also encountered, of varying size, of a gray slate or yellowish color, developed by preference in the anterior lobe of the lung. The tissues quickly undergo purulent or caseous softening. At times the lobe is solidified into a compact gray mass, sown with irregular cavities, which are filled with fetid muco-pus and formed from dilations of small bronchi or terminal bronchioles.

"In some cases, the anterior lobe of the lung is collapsed and violet red; the tissue, engorged with blood, has become closed to the inspired air by reason of the obstruction of the principal bronchus. Certain lobules of the region shut off are invaded by small rounded swellings, yellowish white, fluctuating, arranged like grapes along the central bronchus and due to dilatations of the bronchi by a sticky muco-pus."

Lesions of the trachea and of the larynx are rare in cattle. When they exist they take the form of a crop of granulations or of more or less deep ulcerations (*Plate XIV*).

B. DIGESTIVE TRACT AND ABDOMINAL VISCERA

The tongue is at times the seat of true tuberculous ulcerations surrounded by hard thickened tissue. The same is true of the tonsils. The sub-lingual or retro-pharyngeal glands are then enlarged and filled with caseous tubercles.

Lesions of the stomach are very rare. They are however observed at times, particularly in the lining of the true stomach, in the form of ulcers which are usually isolated, with elevated fibrous borders, and covered over with grayish muco-purulent material.

The small intestine, especially in the last portion or near the caecum, presents most often conglomerate tubercles in Peyer's patches or the lymphoid glands. Their caseous softening gives place to more or less extensive ulcerations which are ragged and projecting at the edge and concave at the center. The latter is filled with a thick, dirty gray, adherent slime. The under-lying tissues are hard, infiltrated and fibrous. The glandular chains along the whole length of the concave margin of the intestine, between the layers of the mesentery, form elongated large movable masses, which on section reveal a large number of grayish white tubercles with caseous centers.

The liver is frequently the seat of multiple lesions, at times in the form of caseous foci. These vary in size from that of a lentil to that of a hazel-nut, and are of a gray or yellowish color. At times they may form masses as large as an orange, filled with thick, caseous, grumous, odorless pus and ordinarily surrounded with a capsule of sclerosed tissue (*Plates XV and XVI*).

The spleen, which is involved more rarely, shows at times collections of small abscesses of varying size, disseminated throughout the organ.

The kidney may be invaded throughout its parenchyma by miliary tubercles which at times may coalesce to form cavities with caseo-calcareous contents.

C. SEROUS MEMBRANES

Tuberculous lesions of the pleura and of the parietal peritoneum assume a quite distinctive aspect in cattle (perlsucht of the Germans, maladie perlée). They appear first in the form of small granulations in isolated groups or in sheets of a grayish white color, scattered

PLATE XIV

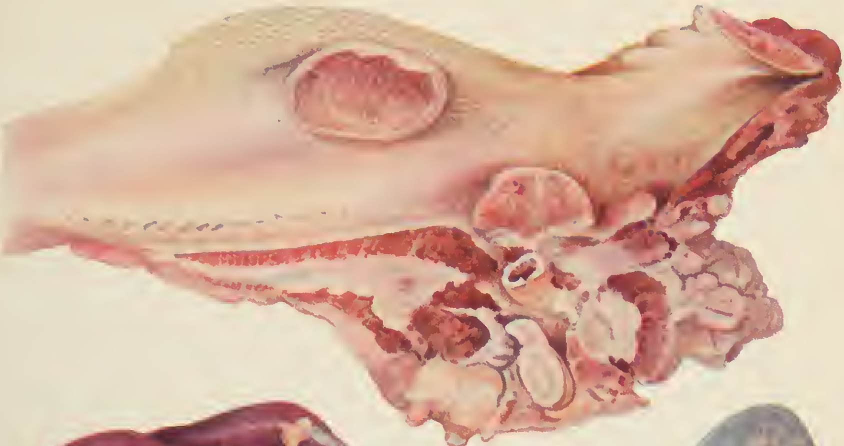
1. Sub-mucous tuberculous ulcer of the tongue of an ox (from an anatomical specimen of P. Chaussé).
2. Tuberculous liver from an ox.
3. Tuberculous spleen from the same ox.

PLATE XV

1. Fibro-caseo-calcareous tuberculosis of the lung in the ox (from a specimen in the Anatomical Museum of the Veterinary School at Alfort)..
2. Miliary tuberculosis in a heifer (from an anatomical specimen in the Bureau of Animal Industry at Washington).
3. Intestinal tuberculosis in the ox (ulceration of the small intestine) (from a specimen in the Anatomical Museum of the Veterinary School at Alfort).

PLATE XVI

1. Fibro-caseo-calcareous tuberculosis of the lung in the ox (abattoir of Lille).
2. Tuberculosis of the rumen in the ox (from an anatomical specimen of M. Chaussé).
3. Tuberculosis of the ovary in the cow (from an anatomical specimen in the Bureau of Animal Industry at Washington).



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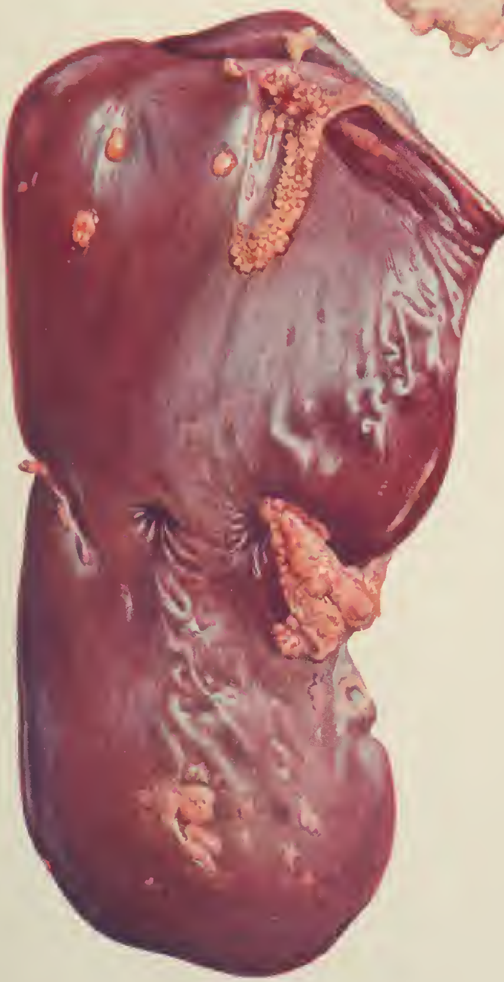

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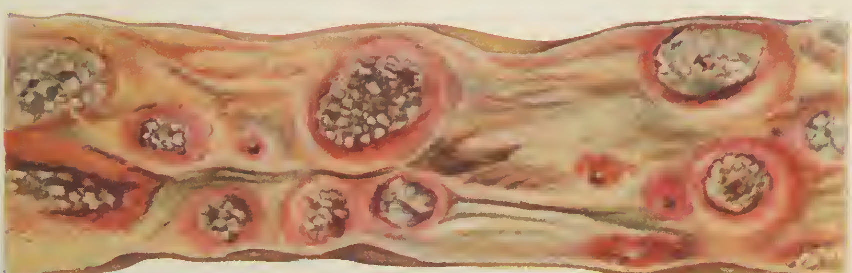

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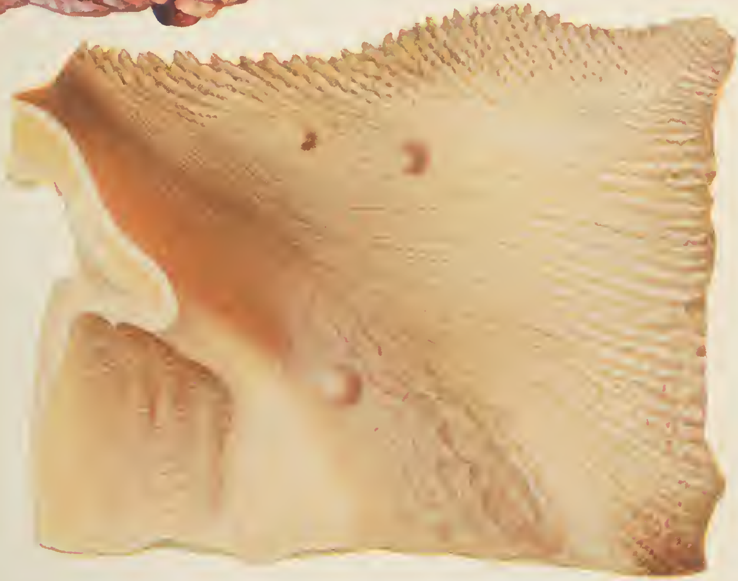

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through the thickness of the serous membrane and resembling a rough leather. Little by little these granulations, developing in the sub-serous spaces, become more compact. They become isolated in small masses or clusters which are prominent, and of a pink color, and which are attached in each case by a distinct pedicle and resemble a collection of polyps more or less rubbed flat. They are often infiltrated with calcareous salts or become fibrotic and resist the knife (*see Plate XIII*).

The pericardium may be the seat of similar lesions. Its layers thicken, and at times become adherent, either entirely or in part (cardiac symphysis).

D. LYMPHATIC GLANDS

The various gland groups participate either primarily or secondarily in the tuberculous infection. The glands affected are often very large in size and hard, with uneven surface, and filled with caseous and calcareous matter which resembles mortar and in which bacilli stainable by Ziehl are found only exceptionally. On section the greyish brown succulent tissue shows large agglomerated tubercles or multiple small yellowish granulations, hard as millet seeds, crushing with difficulty and surrounded by fibrous tissue.

Occasionally no lesions are to be seen, the glands being simply enlarged, or even atrophied, in a state of fibrous degeneration. They nevertheless contain bacilli, even though the latter are not found on direct examination, and inoculation of ground up material into guinea pigs is regularly followed by infection of these small animals.

E. SKIN

Tuberculosis of the skin is regarded as very rare in cattle. There are only some 20 reported cases. Ch. Perard and G. Ramon[3] studied 6 of them in which the infection was located principally in front of the shoulder. They had the form of large painless swellings, varying in size from a pea to that of a hen's egg and containing a cheesy substance in the center with traces at times of calcification. The corresponding glands are at times tuberculous, or again free from involvement. Inoculations indicate that the bacilli contained in these lesions have but a low degree of virulence in comparison with bacilli isolated from human lupus.

[3] Bull. Soc. centr. de méd. vétér., 1913, 91, 167.

F. TUBERCULOSIS OF THE UDDER

This localization of tuberculosis is particularly grave for the animal and from the point of view of the spread of the disease its importance is very great.

As a rule it involves only one quarter of the organ but may invade several, the posterior quarters by preference.

It takes the form of a more or less dense swelling, with hard nodules like small stones, and is always accompanied by a very characteristic engorgement of the corresponding supra- or retro-mammary glands. The engorgement may be considerable.

If the animal is lactating, the milk gradually becomes serous and yellowish. After preserving a normal appearance during a few weeks or even months small purulent lumps extremely rich in bacilli, appear in the milk. At times as many as 100,000 bacilli per cubic centimeter of milk, and 100 per 1 gm. of the butter are found (A. Ostermann).[4]

On section, the infected udder is found full of small, more or less calcified foci which are separated by areas of healthy tissue. At times it is thickly invaded by granulations. True cavities may even be found (*Plate XVII*).

According to Nocard and Leclainche, the histological changes in the tuberculous udder are the following:

"The connecting supports, very thin in the healthy gland, even during lactation, are very dense; the capillaries are distended; many migratory cells infiltrate the connective tissue. According to MacFadyean,[5] it is these lesions which usually initiate the invasion of the organ. The first alterations in the parenchyma seem to be purely mechanical. The interstitial infiltration compresses the lobules and effaces the lumina of the acini and ducts: the result is a retention of contents at different points with cystic dilatations. In a more advanced stage, the epithelium is rolled back and detached by interstitial infiltration and tumbles into the acini where it is found mixed with many round cells. Bacilli in the beginning are demonstrated only with difficulty, but a little later, when giant cells are present and the acinus is altered, they are present in large number, either free or contained in round and epithelioid cells.

[4] Ztschr. f. Hyg., 1908, **60**, 375; 410.

[5] Lancet, 1899, ii, 849;—München. med. Wchnschr., 1891, **38**, 690.

"Destruction of the gland progresses slowly, with complete fibrous transformation. In the sclerosed portions, amorphous masses (amyloid according to MacFadyean, casein according to Moser) fill the lumina of the acini and ducts. The vessels, which are dilated and varicose during the early stages, become compressed and atrophied about the calcified tubercles. Neighboring tissues however contain a rich network of dilated capillaries.

"The villous new growths in the milk ducts are preceded by an epithelial desquamation and an infiltration of round cells in the submucous layer. The villi are formed of granulation tissue identical with that found in ordinary wounds. They undergo a necrosis of their tips and the necrotic elements are cast off into the sinuses. Similar alterations are found in the large milk ducts.

"The milk secreted by the diseased gland has a higher water content than normal, it contains no lactose and only a very little fat (Storch)."

G. GENITAL ORGANS

The testicles, the penis or the vagina may be the seat of tuberculous nodules developed in the sub-serous or the sub-mucous lymphatic spaces. In the cow very extensive lesions are at times found in the ovary. The latter is then large, with uneven surface, and sown with caseo-calcareous or purulent masses. The tubes are almost always invaded, being studded with granulations of a whitish gray color and more or less confluent (*see Plate XVI*).

The infection may also extend to the sub-mucous tissue of the uterus, and if the tubercles go on to softening, the whole organ becomes filled with yellowish, grumous, muco-pus, rich in bacilli.

H. OTHER LOCALIZATIONS

Tuberculous lesions of the nervous centers are encountered with some frequency in cattle, principally in the pia mater about the median fissure and sylvian artery (Nocard) and in the sub-arachnoid lymphatic spaces, even into the hemispheres, the cerebellum (Gassner) and upon the surface of the ventricles.

Moussu[6] reported an observation of tuberculous encephalitis. The lesions spared the meninges, only to localize on the right side in three foci, situated in the parieto-temporal lobe, the frontal lobe, the optic thalami and striated bodies.

[6] Rec. de méd. véter., 1898, p. 737.

PLATE XVII

1. Tuberculosis of the mammary gland in the cow.
2. Longitudinal section of an udder containing a tuberculous cavity.
3. Transverse section of the same.
4. Transverse section of tuberculous oesophageal glands in the cow.

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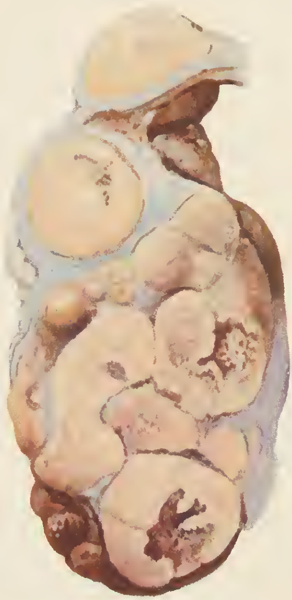

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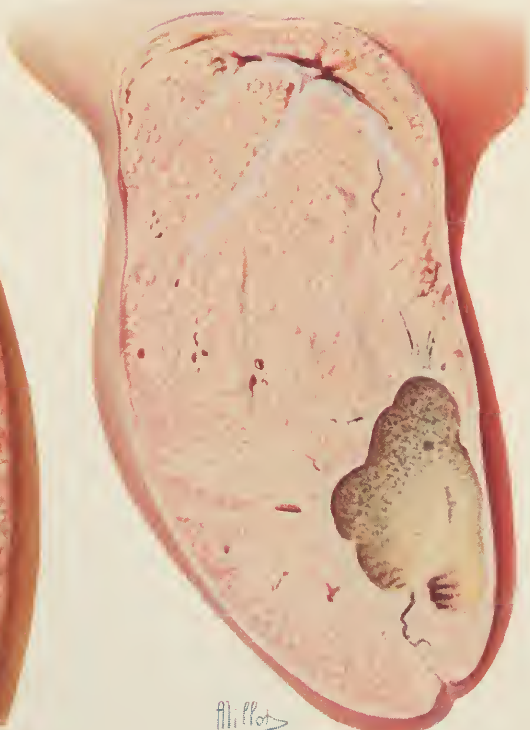

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Ocular tuberculoses have also been observed invading the iris and afterward the choroid and having the appearance of sarcomatous or caseo-calcareous masses, enveloped in fibrous tissue.

The bones likewise may be attacked in cattle as in man, particularly the spongy tissue of the extremities of the long bones, and the synovial membranes of the joints, which now and then show tuberculous lesions. But these localizations are extremely rare and are encountered only in animals with very extensive or generalized tuberculosis.

I. TUBERCULOSIS IN THE CALF

Tuberculosis in the calf exhibits quite special characters which have been carefully studied by Césari,[7] and later by P. Chaussé[8] from the point of view of their pathological anatomy. Glandular reactions are, as a general rule, more intense, and there is a greater tendency to hypertrophy than in the adult animal. Vegetations of the serous membranes also progress more rapidly. In the pleura, as in the peritoneum, they are of a red or yellowish color, flattened out, with rounded margins extending beyond the pedicles of insertion; simulating a macaroon, but the lesions are unequal in size. On incision these macaroons are not caseous, or are barely so. At times one is surprised to see certain cases of congenital tuberculosis with peritoneal localizations already very numerous and highly developed soon after birth. The vegetations, which are at first distinct, may fuse to form a uniform layer of tissue containing bacilli. This layer may be 15 to 20 mm. thick and reflected over a part of the diaphragm or parietal peritoneum. Histologically there are no distinct tubercles, but there are giant cells and the outlines of tubercles disseminated in the hypertrophied glandular tissue. These giant cells are characterized by frequently containing a degenerated "hemateinophile" protein matter which is not encountered in the adult animal. The substance which possesses this affinity for hematein is found when calcification first begins, and in all probability the calcareous salts are responsible for the fixation of the color (Chaussé).

In Morel's[9] opinion tuberculosis of young calves fairly frequently originates by way of the umbilicus and results from contamination of the umbilical wound by infectious excrement in the stable.

[7] Rev. gén. de méd. vétér., 1904, Oct.

[8] Rev. de path. comparée, 1914, March, p. 22.

[9] Ibid., 1914, March, p. 54.

CHAPTER XXIII

FREQUENCY AND GEOGRAPHIC DISTRIBUTION OF SPONTANEOUS TUBERCULOUS INFECTION IN CATTLE

In all countries where cattle are kept stabled for long periods or permanently, tuberculosis is very widespread. Contrariwise it is uncommon or even exceptionally rare in tropical countries and wherever animals live in the open on pasture lands. But colonization at the present time tends to diffuse it almost everywhere through the introduction of animals from contaminated regions for crossing with tuberculosis-free native races. This is the case with the herds of the Argentine and of the Island of Madagascar which escaped contagion up to a few years ago but are now beginning to be so seriously affected that rigorous measures on the part of the sanitary authorities have become necessary.

We have no accurate information on the percentage of the French cattle which are tuberculous. E. Leclainche[1] thinks that Champagne, Lorraine and Brie are infected to a high degree. In the Vosges, 40 per cent of milch cows react to tuberculin. In Brittany, Nivernais, and particularly in the Southeast, the proportion is still higher; in certain valleys of the Pyrenees it reaches 50 per cent of the herds.

An investigation, conducted in 1910 by my collaborator C. Guérin among the directors of the veterinary sanitary services of the various departments of France, brought out information which is quite reassuring. It shows that in the whole of the country the average proportion of animals reacting to tuberculin is approximately 16.5 per cent. The departments most seriously infected are:

	per cent of cattle tuberculous
Landes	30–35
Ardèche	30
Haute-Savoie	30
Charente-Inférieure	30
Haute-Loire	25

[1] Rev. de la tuberc., 1896, **4**, 301.

	per cent of cattle tuberculous
Puy-de-Dôme	25
Ardennes	25
Dordogne	20
Deux-Sèvres	20
Saône-et-Loire	20
Oise	20
Meurthe-et-Moselle	15
Nièvre, Ain, Vosges, Côtes-du-Nord, Morbihan, Finistère, Aisne, Aude	10

According to statements furnished us by Monsarrat, director of the departmental veterinary service, the total number of cases of tuberculosis in cattle observed in the single department of the Nord during the year 1911 was 4,010 (in 1910 it was 4,288). The financial loss resulting was 324,000 francs.

But if one considers separately the dairies situated in the large cities or near them and which are full of cows made to produce milk intensively, it is found that the proportion of tuberculous animals is very much higher. In many localities it reaches 65, 80 and even 90 per cent.

In Paris, where supervision is relatively better than elsewhere, Martel found 43.79 per cent in 1905.

In Italy, among 552 tuberculin tested cows on the farms of the plains about Milan, Gosio[2] states that 193 or 35 per cent gave positive reactions. In the region of Brescia the percentage is 30.

Great Britain is no better off. The proportion of animals found to have tuberculous lesions in the large abattoirs of London in 1892 was 25 to 40 per cent and the proportion of animals reacting to tuberculin in England from 1897 to 1900 was 25 per cent.

MacFadyean estimates that at least 30 percent of the milch cows in the whole of the country are tuberculous and, at Birmingham, from October, 1907, to October, 1910, John Malcolm found 168 among 554, about 31 per cent.

In the Island of Guernsey off the coast of Normandy, bovine tuberculosis was unknown up to 1916. At that time it was introduced

[2] Bull. de l'Office internat. d'hyg. publique, 1912, **4**, 1380.

with the return of animals sent to an exhibition in England; it is still however very rare.

Of 1364 cattle exported from that island between 1908 and 1911 and which were all tested with tuberculin, only 6 were found infected (H. D. Bishop).[3]

In Germany, according to Ostertag, the average of tuberculous cattle, calves not included, is 25 per cent. At the abattoir of Leipzig in 1900 the figure was 35.29 per cent. Of 259 cattle tested with tuberculin by Klimmer at Dresden in 1903, 79 per cent reacted.

According to abattoir statistics collected by the KK. Gesundheitsamt, in Prussia the proportion of tuberculous cattle was:

	per cent
1904	17.88
1905	19.15
1906	20.66
1907	21.21
1908	20.88
1909	21.09
1910	22.51

The average percentage varies according as one includes in the calculation calves under two years, bullocks, bulls and cows. The following, for example, are the figures for Prussia for these respective categories in the year 1910.

Calves under two years	8.33
Bullocks	23.86
Bulls	20.39
Cows	30.88

Under three months only about one calf in 10,000 is found tuberculous. The proportion is about the same in France.

Tuberculosis in cattle, therefore, increases rapidly with age and is considerably influenced by stabling and prolonged lactation. Animals of about 6 years and especially milch cows are the most affected.

Von Behring in the following table has summed up the average results from statistics as to the percentage of tuberculous cattle at different ages in Denmark, Norway and Marburg:

[3] Brit. M. J., 1912, i, 217.

	0 TO 6 MONTHS	6 MONTHS TO 1 YEAR	1 TO 2 YEARS	2 TO 5 YEARS	OLDER THAN 5
	per cent	per cent	per cent	per cent	per cent
Denmark (Bang) 1896	15.5	29.4	40.5	49.3	
Denmark (Bang) 1898	10.6	19.0	25.6	32.8	
Norway (Malm)	1.0	3.4	7.9		10.2
Germany at Marburg (Behring)		5.32	8.72	14.67	19.09

In Norway, Bentzen[4] reports the proportion of tuberculous cattle as varying from 7.3 to 35.7 per cent according to the district.

In Denmark, Jensen gives the figure as 30 per cent for the abattoir at Copenhagen. The proportion reaches 50 per cent in Belgium (according to Heymans) and in Holland.

In Switzerland the proportion is very irregular. The disease is less widespread in the canton of Fribourg and very common in the cantons of Berne, Zurich and Geneva.

Russia, according to Sommer, has fully a half million tuberculous cattle and probably more. In 1895 Petrowsky found 18.2 per cent reacting to tuberculin on the farms of the military government of Ural.

The proportion is somewhat lower in the United States: 20 per cent according to Schroeder. A. D. Melvin, director of the *Bureau of Animal Industry* at Washington, estimates that 3.5 per cent of American herds are infected.

In certain milk districts in the State of New York, V. A. Moore[5] found in 1907, 28.07 per cent of animals reacting to tuberculin. Of 683 farms, 423 were infected. More than 400,000 tuberculin tests, made throughout the country from 1893 to 1908 by the government service, showed the average proportion of the tuberculous cattle to be 9.25 per cent. Of a total of 23,619 animals reacting, the tuberculin results were confirmed at autopsy in 98.81 per cent of cases.

For Mexico, Fleming puts the proportion of tuberculous cattle at 34 per cent.

In the Argentine, on the other hand, the proportion is said to be barely as high as 0.5 per cent for the native cattle, while it rises to

[4] Bull. de l'Office internat. d'hyg. publique, 1913, **5**, 1343.
[5] *Bovine Tuberculosis and Its Control*, Ithaca, 1913, Carpenter.

25 to 45 per cent for those imported (especially the Durham) for cross breeding.[6]

The same is true for Chili and Peru.

In Africa, tuberculosis is very rare among the native breeds of cattle. In Algeria, the proportion does not exceed 1 in ,10,000; imported cattle however are very often infected. In Egypt, Piot-Bey reports 5 per cent of positive reactions among animals on the Government farms. In the Transvaal, bovine tuberculosis, according to Theiler, is practically unknown. In the region back from the west coast, it exists only in localities where cattle have been imported from Europe for crossings with native stock; this is the situation in Cameroons, for example, at the station of Buca (Ziemann). It is unknown in the Sudan.

In Asia, the same is true of the native breeds of cattle. In Indo-China, for example, neither cattle nor buffalo are ever found tuberculous. This is likewise the case in Japan. But the imported animals, which are largely bred in England and America, react to the extent of 50 per cent.

As to Australia, the statistics of the abattoirs of the State of Victoria give 10 to 20 per cent of cattle as tuberculous.

It may therefore be asserted that, if one excepts the human race, of all mammals spontaneously susceptible to tuberculosis and in all parts of the world where stabling is ordinarily practised, cattle are the most susceptible and the most seriously infected. The economic losses due to tuberculosis are enormous and result not only from the loss in value and condemnation of the diseased animals, but also and chiefly from their lowered capacity for gaining weight and for producing milk. It has been calculated that for Great Britain the figure would reach at least 25 million francs per year (Brittlebank)[7] for the United States 40 million francs (A. D. Melvin).[8] For France it is certainly not lower than 20 million francs per year.

The enormity of these losses imposes upon all civilized nations the obligation of combating such a scourge to the utmost.

[6] Rpt. of Bidart to the Minister of Agric. of the Argentine Republic, Buenos Aires, 1909.

[7] Vet. Rec., 1907/8, **20**, 873.

[8] Internat. Congr. on Tuberculosis, 6th., Wash., 1908; Am. Vet. Rev. 1908, **32**, 206.

The campaign instituted at the present time in various countries is absolutely ineffective. Isolation, and still more the slaughter of positively reacting animals, is impracticable since the animals are too numerous.

Tuberculous infection as indicated by a positive tuberculin reaction is as frequent, or approximately so, in the bovine as in the human race. Now with cattle, as with man, it is impossible and illogical to think of eliminating every animal with a latent or concealed lesion, capable probably of discharging virulent bacilli now and then and of spreading contagion through the excretions, but whose apparent health remains such that the economic value equals that of normal animals.

By Bang's method one will certainly succeed in eliminating tuberculosis from a given farm, as Nocard has shown. The method consists in segregating in a special stable, away from contact with the healthy remainder of the herd, all animals which react to tuberculin, and also in isolating from birth, in order to feed them with sterilized milk, such calves as are born of tuberculous cows. But the procedure is costly and cannot be generally employed. It is applicable only to dairies furnishing milk for children and invalids, and in this way has been used with complete success by the Danish Government.

In France, the system of paying indemnities for the seizure of meat and the killing of tuberculous animals has brought about no improvement in the sanitary conditions of cattle since its adoption in 1898. It imposes upon the national budget a very heavy burden which at the present time exceeds one and a half million francs per year and which unquestionably might be better used to further, for example, the development of mutual insurance organizations against the cattle mortality, so that the breeders themselves would become interested in the sanitation of their stables.

The fight against bovine tuberculosis obviously can succeed only when it shall be possible to make general the use of a method of *preventive vaccination*. In another chapter (XLII) we shall study the many attempts which already have been made with this end in view.

CHAPTER XXIV

THE SPECIFIC DIAGNOSTIC REACTIONS OF BOVINE TUBERCULOSIS

In cattle, as in man, the clinical diagnosis of tuberculous infection is possible only when organic lesions exist such as can be demonstrated by the various exploratory methods. It often happens that these latter are quite incapable of revealing even grave forms of the disease, since abattoir statistics show that about 36 per cent of cases of tuberculosis involving complete loss of the meat pass unrecognized when examined alive.

Therefore, in order to establish a diagnosis one should always utilize, according to circumstances, the direct bacteriological examination, supplemented as far as possible by experimental inoculation into the guinea pig and injections of tuberculin.

Bacilli may be sought for in the lymph nodes, for example, or in closed lesions of the different organs, such as the udder, by the procedure of harpooning (Nocard, Ostertag), which permits the removal and direct examination of smears of tissue. Differentiation from non-pathogenic acid-fast bacilli is necessary when examination is made of nasal discharges as well as of those from the trachea or genital organs, from milk, intestinal contents or dejections. This may be accomplished with all necessary accuracy by subcutaneous inoculation into the guinea pig. But even when this result is negative one is not justified in deducing the non-existence of tuberculosis unless the animal when alive failed to react to tuberculin.

Inversely, as will soon be seen, the absence of reaction to tuberculin does not suffice to rule out the diagnosis of tuberculosis, since grossly tuberculous animals, particularly the cachectic, as a rule no longer react although under such circumstances their lesions teem with bacilli, so that experimental inoculation, or direct examination of the discharge or material scraped from the lesions, gives sufficiently exact information.

A. METHODS OF USING TUBERCULIN

Healthy cattle tolerate large doses of Koch's old tuberculin, one to two grams for example, without any trouble. In those infected with the bacillus, on the contrary, no matter how slightly, 0.2 to 0.5 gm. of this same tuberculin injected subcutaneously is enough to produce an elevation of temperature of from 1 to 2°C. lasting for several hours.

This important finding resulted from the first experiments made, soon after the memorable communication of Robert Koch in 1890, first by Guttmann (of Dorpat), then by Roeckl and Schuetz,[1] Bang and Salomonsen,[2] Lydtin, Bang, Nocard,[3] Hutyra and many veterinarians.

Since then it has been confirmed and no changes are necessary in the propositions formulated by Nocard,[4] in 1892, in the following terms:

"1. Tuberculin exercises an unquestionably specific action upon tuberculous cattle, this action manifesting itself chiefly by a noticeable rise of temperature:

"2. Injection of a large dose (30 to 40 centigrams according to the size of the subject) causes ordinarily, in tuberculous cattle, an elevation of temperature of from 1.5 to 3°C.:

"3. The same dose injected into non-tuberculous cattle causes no appreciable febrile reaction:

"4. The febrile reaction appears most frequently between the 12th and 15th hour after injection, at times by the 9th hour, very rarely after the 18th; it always persists during several hours:

"5. The duration and intensity of the reaction bear no relation to the number and gravity of the lesions; it would even seem that the reaction is more sharp in cases where, in the presence of a very limited lesion, the animal has preserved a healthy appearance:

"6. In grossly tuberculous cattle, phthisical in the proper sense of the word, particularly in those with fever, the reaction may be weak or even absolutely lacking:

"It is wise to take the temperature of the animals both morning and evening several days before the injection. Because of some

[1] Ztschr. f. Fleisch.- u. Milchhyg., 1891, March.

[2] Berl. tierarztl. Wchnschr., 1891, April 6; 9.

[3] Bull. Acad. méd., 1891, **55**, 476; 643.

[4] Ann. de l'Inst. Pasteur, 1892, **6**, 44.

passing disturbance or a slight illness (troubles with digestion, or gestation, heat, etc.), some animals show wide oscillations of temperature which may lead to error; in such a case the test should be deferred. The injection should not be performed on animals in pastures; atmospheric changes (rain, wind, fog, sun) as also transportation, may set up variations in temperature; the subjects to be tested should be kept confined at least 24 hours before injection.

"In some tuberculous cattle without fever, the reaction following the injection of tuberculin scarcely exceeds one degree; nevertheless, since experience has taught that the temperature of healthy animals may vary as much as one degree centigrade, reactions should not be regarded as of real diagnostic value unless higher than 1.4°C. A rise of temperature of less than 0.8°C. is of no significance whatever. Any animal in which the temperature elevation is between 0.8° and 1.4°C. should be held in suspicion and should be retested, after an interval of at least one month, with a stronger dose of tuberculin.

"Injections of tuberculin have no harmful influence upon the health of the animal. *Lactation and gestation are ordinarily not affected,* although fairly often during the few days following a tuberculin reaction, milch cows give a noticeably smaller quantity of milk."

Ostertag believes that one should regard as suspicious every test animal which shows a variation of 0.5°C. as compared with the highest temperature before injection. In the case of calves up to the age of 6 months, a temperature above 40°C. with a rise of at least 0.5° has the same significance (*fig. 23*).

B. TECHNIQUE OF SUBCUTANEOUS INOCULATION OF TUBERCULIN

The tuberculin usually employed in France, and also in many other countries, is that prepared by the Pasteur Institute, which delivers the product for veterinary use in the form of a 1 in 10 dilution in 0.5 per cent carbolated water.

Three to 5 cc. of this dilution represent a single dose for adult cattle, according to the size of the animal, and 1 to 2 cc. are sufficient for calves under one year.

Injection should be made in the evening, preferably at 9 o'clock, in order that the whole of the following day may be available to follow the temperature reaction.

It is done with a 5 to 10 cc. syringe which can be sterilized and which is provided with a good strong steel needle. The site of choice is the middle of the side of the neck or behind the shoulder.

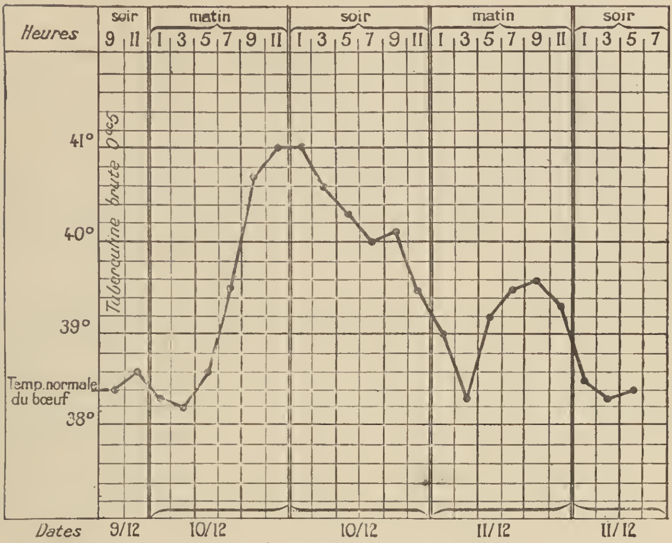


FIG. 23. TYPE OF TUBERCULIN REACTION IN TUBERCULOUS CATTLE

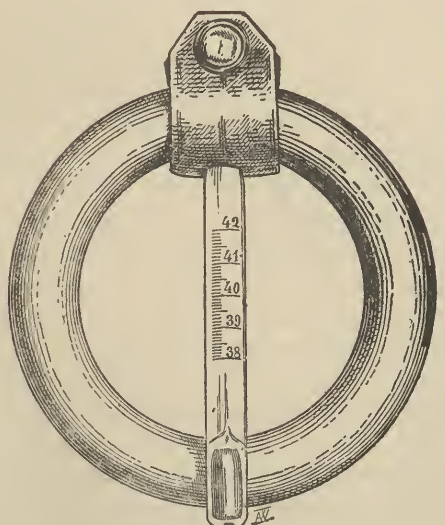


FIG. 24. PESSARY THERMOMETER OF C. GUÉRIN, FOR CONTROLLING TUBERCULIN REACTIONS IN THE COW

It is better to take the temperature every two hours and this practice is always recommended. But if this cannot be done the temperature may be noted at 9 o'clock in the evening (at the time of injection) and the following day, at 5, 7 and 9 o'clock in the morning, at noon, and at 3 and 6 o'clock in the afternoon, that is to say 8, 10, 12, 15, 18 and 21 hours respectively after injection.

The animal should not be allowed to drink during the hour preceding each taking of temperature, since ingestion of a large amount of cold water may considerably lower the temperature of the interior of the body (Nocard).

The highest point of the temperature curve is reached generally from the eighth to the sixteenth hour, but it must be realized that certain animals react more quickly and others more slowly. If great precision is desired one should, in the case of cows, make use of a small self-registering thermometer graduated from 38 to 42° only and mounted transversely on a pessary which is left in the bottom of the vagina. In this way one can be certain that early reactions do not pass unnoticed (C. Guérin)[5] (*fig. 24*).

C. INTERPRETATION OF RESULTS OF INOCULATION OF TUBERCULIN

Every definite reaction which is 1°C. or more above the maximum normal should lead to the conclusion that a tuberculous focus exists; but the latter may be so very small that, on killing and autopsying the animal, it is at times found only with the greatest difficulty. Tuberculin has frequently been accused of having given a false indication because no tuberculous lesion could be found. It was proven however long ago that in these circumstances the organs had not been searched with sufficient care and that, whenever a tuberculin reaction is positive, there exists somewhere a follicular lesion or at least a gland containing bacilli whose presence can be disclosed by experimental inoculation into the guinea pig.

The question arose as to the interval of time between the entrance of bacilli into the body of cattle and the beginning of their susceptibility to tuberculin. To throw light upon this point, experiments were performed separately by Nocard and Rossignol, by a Commission of the *Royal Agricultural Society of England* and by various observers (Dinwiddie, Ward, Marshall,[6] and others). The results—

[5] Bull. Soc. centr. de méd. vétér., 1907, **61**, 281.
[6] Internat. Congr. on Tuberculosis, 6th, Wash., 1908.

as was to be expected—were much at variance in that the experimenters resorted to particularly severe methods of artificial infection and were not working under identical conditions. For example, after infection by way of the digestive tract, Nocard and Rossignol found the first positive reactions from the thirty-second to the forty-eighth day in 4 animals, while the English Commission found them from the eighth to the fifty-first day in 5 animals.

Following infection by inhalation the reaction appeared between the nineteenth and thirty-second day. After introduction of the virus into the udder by means of a milking tube, it was positive on the thirteenth day (Nocard) and after subcutaneous inoculation, from the eighth to the fifteenth day (*English Commission*).

It seems evident that *tuberculin produces a reaction as soon as a follicular tuberculous lesion has formed or as symbiosis is established between the bacilli and the lymphatic cell phagocytes*—as is actually the case in vaccinated animals—as we shall see later.

D. ACQUIRED TOLERANCE FOR TUBERCULIN.—"DOPING."—ITS DETECTION

While it is true that every positive tuberculin reaction indicates the existence of tuberculous infection (active, latent or occult) every negative reaction does not necessarily exclude the presence of tuberculosis.

Animals which, by virtue of the severity of their lesions, are saturated with tuberculin secreted by their own bacilli, as well as those with a hectic fever, or cachectic, or again those which are vaccinated with human bacilli (Lignières[7]), can no longer react. Clinical examination or past history then usually gives the veterinarian enough information and autopsy soon removes any doubt still remaining.

This saturation of the body with tuberculin ("doping") may be artificially brought about from wrong motives, by cattle dealers or dishonest importers wishing to sell or introduce known tuberculous animals into a country or market, and passing them off for healthy. To accomplish this it is only necessary to repeat the injection a certain number of times, when there will be produced a sort of tolerance and the characteristic reactions are no longer observed. But Malm[8]

[7] Bull. Soc. centr. de méd. vétér., 1907, **61**, 90.
[8] Rev. gén. de méd. vétér., 1903, p. 401.

has shown that this tolerance is irregular, temporary and uncertain and that it disappears fairly quickly. Vallée[9] (of Alfort) studied the phenomenon very carefully. He reinjected cattle, which had just given strong reactions, with a second dose of tuberculin and found that, if the temperature be taken every two hours after inoculation, there is observed a rapid and very marked rise of temperature, an elevation which is sufficiently definite in all cases to permit of the rested animals being called at least suspicious. Non-tuberculous subjects on the other hand remain unaffected by this procedure. From his experiments he drew the practical conclusion that a given animal suspected of having undergone a preliminary tuberculin treatment may be detected by testing as follows:

"At 5 or 6 o'clock in the morning inject a dose of tuberculin twice as large as usual (8 cc. of a 1 in 10 dilution for large animals, and 4 cc. for small).

"Take the temperature every two hours from the time of injection up to about the fourteenth or fifteenth hour.

"Reaction is measured by the difference between the temperature at the moment of injection and the highest temperature registered during the following hours. Any animal giving a reaction of 1.5°C. should be regarded as tuberculous; a reaction between 0.8 and 1.5°C. should arouse suspicion."

By observing the effects on temperature of small, constant doses of the same product (0.4 gm. of Merck's tuberculin), administered at long intervals of at least 2 months Hauptmann found that, in general, sensitiveness disappears after the second or third injection to reappear again only after a rest period of 7 to 10 months.

E. LOCAL REACTIONS TO TUBERCULIN

At the present time the fraud of *doping* in cattle may be very easily detected by successively instilling tuberculin into the conjunctiva, inoculating it intradermically, and then injecting it subcutaneously; in other words, by performing the three tests on the same animal, the *conjunctival, intradermic* and *subcutaneous*. Even though an animal has been repeatedly injected subcutaneously, tuberculin placed upon the ocular mucous membrane or introduced

[9] Ann. de l'Inst. Pasteur, 1904, **18**, 545.

into the dermis with a needle will still produce its characteristic effects (*see Chapter XXXVI*).

Instillation into the eye (*ophthalmic* or *ocular test*) was being employed in cattle by Vallée at the same time that Wolff-Eisner and Calmette were making known their results with this method for the diagnosis of tuberculosis in man. Lignières in the Argentine, White and McCampbell[10] in the United States, and Klimmer and Kiessig in Germany, utilized it somewhat later.

The technique is very simple. One of the upper lids is raised and pure tuberculin introduced underneath by means of a fine badger-hair brush. The eye is then gently rubbed to spread the tuberculin between the lid and the sclera.

If the hair pencil is not available, approximately 0.1 cc. of tuberculin may be put into the open eye with a medicine dropper.

After 5 or 6 hours the lids begin to swell slightly and after about 16 or 17 hours the capillary network of the nictitating and sclerotic membranes is congested and the membranes themselves assume a very distinctive reddish purple color. The eye lachrymates and the conjunctiva becomes covered with a grayish white exudate containing many polynuclear leucocytes. On comparing it with the untreated eye the diagnosis can be read immediately. Reaction disappears from the third to the sixth day and leaves no trace.

C. Guérin and A. Delattre,[11] and later Morel, called attention to the curious fact that if one injects tuberculin subcutaneously into cattle which have previously been tested with the ophthalmic reaction, there is a reappearance of redness and lachrymation 12 hours later in the tested eye, just as though a new instillation had been made.

Vallée[12] showed moreover that if one repeats the ophthalmic reaction on the same animal and the same eye, there is observed, in animals which reacted the first time, a true sensitization of the eye tested. In one experiment where four instillations were made in the course of 19 days, the fourth response (one-tenth of a drop of raw tuberculin) was at least as strong as the first (one drop of the same tuberculin). The untested eye preserves its original sensitiveness. In animals however which have not reacted to the first instillation, repeated attempts neither sensitize nor cause the reaction to appear—no more than in healthy cattle.

[10] J. Exper. Med., 1908, **10**, 232.

[11] Bull. Soc. centr. de méd. vétér., 1907, **61**, 375.

[12] Compt. rend. Acad. des sci., 1908, **146**, 416.

Since 1907 the ophthalmic reaction has been quite generally employed by veterinarians who agree as to its great value in the diagnosis of tuberculosis in cattle. Their observations show that although, as with the subcutaneous injection, failure to react does not always exclude the presence of tuberculous lesions, it is nevertheless certain that all animals which do give a positive conjunctival reaction are tuberculous (Sekyra, Irr and Claude,[13] Koehl,[14] Abel,[15] Walter Assmann,[16] Trotter, and others).

The intradermic reaction, first recommended by Moussu and Mantoux[17] is equally reliable for diagnosis. In performing it 1 to 2 centigrams of tuberculin diluted in 10 times its volume of physiological salt solution are injected into the dermis, with care not to introduce the point of the needle into the subcutaneous tissue (*Plate XVIII*).

The site where the reaction can be most favorably observed in cattle, is the middle part of one of the two lateral cutaneous folds which extend from the base of the tail to the margin of the anus. At this point the skin is soft, elastic, free from hair and provided with a very abundant and elastic subcutaneous connective tissue. In pigs the site of election is the antero-superior angle of the ear, over the fold in the cartilages.

Twenty-four hours after injection the result is already very well defined and can be easily read. Maximum intensity is reached at about the forty-eighth hour. If the result is positive, one needs only to raise the tail slightly to see that the fold on the test side is doubled or tripled in thickness, while that of the opposite side has remained exactly as in the beginning. The edematous infiltration takes a violet red tinge and an ovoid shape about the point of insertion of the needle and becomes as large as a hazel-nut or even larger. After the 4th day the reaction disappears. Now and then, in animals which have reacted positively, a small superficial scar is formed which becomes covered with a brownish crust and heals fairly quickly.

Moussu states that the intradermic test performed during the days preceding a subcutaneous injection of tuberculin, does not impair the

[13] Bull. Soc. centr. de méd. vétér., 1907, **61**, 525.

[14] Berl. tierarztl. Wchnschr., 1909, Feb. 4.

[15] Ibid., 1911, **27**, 236.

[16] Ibid., 1911, **27**, 449.

[17] Compt. rend. Acad. des sci., 1908, **147**, 502; Bull. Soc. centr. de méd. vétér., 1908, **62**, 500.

effects of the latter; but on the contrary a subcutaneous injection (which corresponds to a massive dose if compared with that utilized for the intradermic) hinders the development of a local reaction when the intradermic test is being done during or immediately following subcutaneous injection.

But there is no contra-indication to performing the intradermic and the ophthalmic reactions simultaneously in the same animal and if these tests are both negative and the animal still held in suspicion, the subcutaneous injection of a large dose of tuberculin will come in very handily a few days later in removing any doubts which may persist.

In cows, one may go further and utilize the vaginal reaction which Richter[18] (of Dresden) finds to be very practical. It is performed by rubbing pure tuberculin into the folds of the vulva with the thumb. After 24 to 48 hours a slight redness appears, which is soon followed by a fairly marked exudation and some swelling.

These various local reactions are not to be regarded as being so constantly reliable as the thermal reaction after subcutaneous injection, but they are often superior in that they neither affect the general health of the animal nor lessen the milk secretion, and that they call for no special precaution. Their technique is extremely simple. Their results are easily interpreted and if necessary they may be employed in cattle with febrile affections. On these several accounts they render great service.

F. SERO-DIAGNOSIS

S. Arloing and P. Courmont have applied their method of sero-diagnosis to the detection of tuberculosis in cattle, but this laboratory procedure (*Chapter XXX*) is much too delicate to be generally practical. Panisset moreover showed that it was impossible to draw any useful conclusions, since 60 per cent of healthy animals gave positive reactions and the result was negative in 25 per cent of tuberculous animals.

[18] Ztschr. f. Infektionskr. . . . d. Haustiere, 1909, **5**, 243.

PLATE XVIII

1. Intradermic reaction to tuberculin, at the site of election, the left subcaudal fold, in the cow (from Moussu and Ch. Mantoux).

2–3. Intradermic-reaction to tuberculin, at the site of election in swine (from Moussu and Ch. Mantoux).

II

III

I

CHAPTER XXV

RÔLE OF BOVINE TUBERCULOSIS IN THE INFECTION OF MAN

The Question of Milk

In an earlier chapter (XXI) we have discussed the important question first raised by Th. Smith, and then particularly by Robert Koch in his memorable communication at the Congress of London in 1901, *on the duality of human and bovine tuberculoses.* We now accept the conclusion, confirmed by the majority of recent works, that *there exists only one race of tubercle bacilli in mammals* (*Bacillus tuberculosis mammalium*) and that its adaptation to the body of man or cattle—man and cattle being the two species of mammals most susceptible to spontaneous tuberculous infection—has brought about its separation—not absolute however—into two types of bacilli presenting certain differential characteristics: the *human type* and the *bovine type.*

Consequently we shall not again take up this discussion but, from the practical point of view which interests us at present, we need to bear in mind the fact that bacilli derived from tuberculous lesions of cattle and showing the various properties of the bovine type may infect man, although they are in general less virulent for the latter than is the bacillus derived from human tuberculous lesions.

On the basis of the differential characters already described for determining the *bovine* or *human* nature of the different forms of tuberculous infection observed in man, we see on the one hand the extreme rarity of the bovine type in chronic pulmonary tuberculosis of adults and on the other hand its relative frequency in glandular lesions and in the acute infections of childhood.

Thus of 108 cases of tuberculosis of all sorts—lupus excluded—the *English Royal Commission,* whose final report was published in 1911, recognized the human type 84 times, the bovine type 19

times, and a mixture of the two in 5 instances. The cases were divided as follows:

14 pulmonary tuberculoses (examination made of the lesions of the lung and tracheo-bronchial glands)	14 times human type
28 pulmonary tuberculoses (repeated examinations of sputa)	26 times human type 2 times bovine type
3 generalized tuberculoses	3 times human type
3 cases of tuberculous meningitis	3 times human type
5 glandular tuberculoses (bronchial and mesenteric)	3 times human type 2 times human and bovine associated
9 glandular tuberculoses (cervical and axillary)	6 times human type 3 times bovine type
29 intestinal tuberculoses	13 times human type 14 times bovine type 2 times human and bovine associated
14 bone or joint tuberculoses	13 times human type 1 time human and bovine associated
3 tuberculoses of genito-urinary organs	3 times human type

In 20 *cases of lupus* the same *English Royal Commission* found in one instance a type of bacillus corresponding exactly to the bovine, and twice a well defined human type; 8 cases yielded strains which culturally had the appearance of the bovine type but were much modified in their virulence for the bullock and the rabbit. The strains in the 9 remaining cases had the appearance of human bacilli but were of lower virulence than the latter for the monkey and guinea pig.

At Berlin, among 28 cases of lupus directly cultured at the Rudolf Virchow Hospital by Rothe and Bierotte,[1] the human type was recognized 23 times, or in 82.1 per cent; the bovine type in 4, or 14.3 per cent, and a mixture of the two types in 1 instance, or 3 per cent.

From 1912 to 1916, Lydia Rabinowitsch[2] studied 20 cases of tuberculosis in which bovine origin might be presumed: 11 cases of abdominal tuberculosis, of which 7 gave bovine bacilli; 7 cases of glandular tuberculosis, of which 2 were bovine; 2 cases of pulmonary tuberculosis, of which 1 gave bovine bacilli. Among the 20 cases therefore, bovine bacilli were found 10 times (50 per cent), and if one takes into account only cases of tuberculosis in children, the percentage of tuberculoses of bovine origin is still higher (70 per cent).

Griffith[3] in England, in his most recent investigations found 13 cultures of human type in 25 cases of lupus, while the 12 others presented the characters of the bovine type.

Of 281 cases of tuberculosis in children observed at Edinburgh, Chung Yik Wang[4] isolated the bovine bacillus 80 times among 102 patients under 5 years, and 45 times among 64 patients of from 5 to 16 years. Of children bottle-fed with raw cow's milk, 37.5 per cent gave a positive tuberculin reaction. Among those fed with boiled milk, only 14.5 per cent reacted.

At Paris, Et. Burnet[5] isolated the human bacillus from 31 cases of glandular tuberculosis, from 11 cases of joint tuberculosis and from 16 cases of cutaneous tuberculosis. Not once did he find the bovine bacillus. Almost all of these cases were children. These results indicate that infection of bovine origin is very rare among the population of Paris.

Further statistics have been collected in Germany, Denmark, Sweden and the United States. Park and Krumwiede[6] collected in 1911 a total of 1224 observations made in different countries and

[1] Veröffentl. d. R. Koch Stift. z. Bekämpf. d. Tuberk., 1913, H. 8/9, 87.
[2] Berl. klin. Wchnschr., 1917, **54**, 77.
[3] J. Path. & Bact., 1914, **18**, Suppl., 591.
[4] Edinburgh Med. J., 1917, **18**, 178; 315.
[5] Compt. rend. Soc. de biol., 1914, **76**, 416.
[6] J. Med. Research, 1910, **23**, 205; 1911, **25**, 313.

in which were given the bovine or human type of the infection and the age of the subjects. The following is their table:

DIAGNOSIS	ADULTS OF MORE THAN 16 YEARS		CHILDREN FROM 5 TO 16 YEARS		CHILDREN YOUNGER THAN 5 YEARS	
	Human	Bovine	Human	Bovine	Human	Bovine
Pulmonary tuberculosis	644	1	11		23	1
Glandular tuberculosis (axillary or inguinal)	2		4		2	
Cervical gland tuberculosis	27	1	36	21	15	21
Abdominal tuberculosis	14	4	8	7	9	13
Generalized tuberculosis (alimentary origin)	6	1	2	3	13	12
Generalized tuberculosis (with meningitis, alimentary origin)	29		5	1	46	13
Generalized tuberculosis (with meningitis)	5		7		52	1
Tuberculous meningitis	1		3		27	4
Bone and joint tuberculosis	27	1	38	3	26	
Genito-urinary tuberculosis	17	1	2			
Cutaneous tuberculosis	3		1		1	
Other tuberculoses: Of the tonsils				1		
Of the mouth and glands of the neck		1				
Of the maxillary sinus	2					
Latent tuberculosis					1	
	777	10	117	36	215	65

Mixed infections (bovine and human): 4 cases. Total: 1224 cases. The percentage of cases of bovine infection, mixed infections excluded, is therefore:

	ADULTS OF 16 YEARS AND OVER	CHILDREN FROM 5 TO 16 YEARS	CHILDREN UNDER 5 YEARS
	per cent	*per cent*	*per cent*
Pulmonary tuberculosis	0	0	4.1
Glandular tuberculosis	3.6	36.0	58.0
Abdominal tuberculosis	22.0	46.0	59.0
Generalized tuberculosis	2.7	40.0	23.0
Tuberculous meningitis	0	0	13.6
Bone and joint tuberculosis	3.5	7.3	0

Park concludes that the bovine type of bacillus is a negligible factor in so far as infection of adults is concerned. It but rarely causes phthisis.

This is also the conclusion of H. Kossel,[7] who has assembled in the following table the results published by 27 investigators.

Type of tubercle bacillus isolated from sputa in chronic pulmonary tuberculosis in man

NUMBER	INVESTIGATORS	NUMBER OF CASES	HUMAN TYPE ALONE	BOVINE TYPE ALONE	ASSOCIATION OF THE TWO TYPES	INDEFINITE TYPE
1	Th. Smith (America)	6	6			
2	Vagedes (Germany)	6	6			
3	Kossel, Weber and Heuss (Germany)	9	9			
4	De Jongh, Steuermann (Holland)	2	1	1		
5	Arloing, Pupier, (France)	2	2			
6	Link (Germany)	1	1			
7	Henschen, Jundell and Svensson (Sweden)	2	1			
8	Dammann and Müssemeier (Germany)	1	1			
9	Fibiger and Jensen 1905 (Denmark)	10	10			
10	Fibiger and Jensen 1910	10	10			
11	Gorter (Holland)	21	21			
12	Mohler and Washburn (U. S. A.)	9	8			1
13	L. Rabinowitsch (Germany)	5	5			
14	English Commission (second report)	2	2			
15	Zwick (Germany)	1	1			
16	Meyer (Germany)	2	2			
17	Dieterlen (Germany)	50	50			
18	Kitasato (Japan)	152	152			
19	Park and Krumweide (U. S. A.)	296	296			
20	Jancso and Elfer (Hungary)	5	5			
21	English Commission (final report)	28	26	2		
22	H. Kossel (Germany)	46	45		1	
23	Möllers (Germany)	51	51			
24	Bulloch (England)	23	23			
25	Weber and Dieterlen (Germany)	9	9			
26	Lindemann (Germany)	41	40		1	
27	Gosio (Italy)	42	42			
	Totals	832	826	3	2	1

[7] Veröffentl. d. R. Koch Stift. z. Bekämpf. Tuberk., 1913, H 8/9, 1.

On the other hand, 6 to 10 per cent of deaths from tuberculosis below the age of 5 years are to be attributed to the bovine bacillus, and the latter is frequently the causative factor in glandular lesions and generalized infections, particularly in children.

According to Kossel, the bovine bacillus is found in only 4.3 per cent of cases of bone tuberculosis, while it is much more frequent in meningitis (10.7 per cent) and generalized tuberculosis (23.8 per cent), and still more so in cervical adenitis (40 per cent) and abdominal tuberculosis (49 per cent).

A special investigation was made by A. Philip Mitchell (of Edinburgh)[8] as to the origin of cervical adenitis in 72 children. The results were as follows:

AGE	TYPE OF BACILLUS		TOTAL
	Human	Bovine	
From 0 to 1 year	2	1	3
1 to 2 years		16	16
2 to 3 years		8	8
3 to 4 years	1	10	11
4 to 5 years		4	4
5 to 6 years	1	4	5
6 to 7 years		5	5
7 to 8 years		5	5
8 to 9 years		5	5
9 to 10 years	1	4	5
10 to 12 years	2	3	5
Totals	7	65	72
	or 10 per cent	or 90 per cent	

In this series 84 per cent of the children under 2 years had been fed from birth with unsterilized cow's milk.

Further, J. Fraser (of Edinburgh)[9] studied 67 cases of bone and joint tuberculosis and found bovine bacilli 42 times, human bacilli 22 times and mixed infection 3 times. Of 47 children under 5 years there were 32 bovine infections, 12 human and 5 mixed. The younger the children, the greater was the predominance of the bovine type.

Of 4 tuberculous nurslings under one year, not one showed the

[8] Brit. Med. J., 1914, i, 125.
[9] J. Exper. Med., 1912, **16**, 432.

human type. Of 43 bottle-fed infants, 35 presented the bovine type, 3 both types, and 3 the human type alone. In breast-fed infants, on the contrary, in 24 cases the human type was found 19 times and the bovine type 7 times. Of 46 children from families where there had been no previous tuberculosis, 43 showed the bovine bacillus, whereas in 21 cases occurring among children of tuberculous parents, only 6 were of the bovine type.

Among 171 children of from three weeks to twelve years of age, also affected with cervical adenitis, Ungermann[10] finds that in 76 per cent of the cases other gland groups are likewise the seat of infection, although healthy in appearance. This goes to prove that the penetration of the bacillus into the blood stream occurs early and precedes localization in the cervical glands. Ungermann was able to isolate the bovine bacillus only twice (1.16 per cent). In all the other subjects the human type was found.

A. de Besche,[11] of Christiania, considers that 6 to 8 per cent of human infections have their source in cows. From 50 cases of tuberculosis in children taken at random, he isolated pure human bacilli in 45 instances, pure bovine bacilli in 3, a mixture of the two types in 1, and in the single case remaining, an undetermined type. The gravity of the lesions to all appearances was the same. To his mind there was no reason therefore to judge that the bovine bacillus is less virulent for man than is the human type.

From 40 cases of tuberculosis in nursing infants Karl Stefenhagen[12] isolated the human bacillus 35 times from the various gland groups, and the bovine bacillus only 5 times.

In conclusion, Et. Burnet[13] at Metchnikoff's laboratory, at the Pasteur Institute, did not find a single bovine strain among 59 races isolated from external tuberculoses. Of 24 gland cases which he studied—all Parisians—3 were under 5 years of age; 6 from 5 to 16 years; 2 from 17 to 21 years and 3 were adults.

It would seem therefore that the frequency of infection by the bovine bacillus varies in different countries, and that in Paris, for example, it is much less than in London and New York.

Such a discordance in the results of examinations carried out with practically identical methods and based generally upon the trial of

[10] Tuberk.-Arb. a. d. k. Gsndhtsamte, 1912, H. 12, 109; 213.

[11] Deutsch. med. Wchnschr., 1913, **39**, 452.

[12] Tuberk.-Arb. a. d. k. Gsndhtsamte, 1912, H. 11, 25; 52.

[13] Ann. de l'Inst. Pasteur, 1912, **26**, 868.

virulence for the rabbit, indicates how difficult it is to distinguish positively the human from the bovine type. In fact O. Malm[14] was quite correct in stating that it is hardly possible to determine the origin of a given strain with absolute certainty on the basis of its virulence; bacilli vary enormously according to the media on which they are grown and according to the animal species into which they are inoculated.

From the sum total of facts collected by investigators in the course of recent years, the conclusion apparently should be drawn that tuberculosis of bovine origin is dangerous for man chiefly during early life.

If it is true, as an observation by Weber and Stefenhagen[15] would indicate, that, in bone tuberculosis of children, the bovine bacillus retains its type characters of culture and virulence for several consecutive years (4 years in the case cited by these authors), it is possible, as we have stated earlier, that the practically exclusive finding of human type bacilli in adult pulmonary tuberculosis is the result of a progressive adaptation to the body on the part of the infecting bacillus, originally bovine in type; that these originally bovine bacilli, having gained access to the human body in the early years of life, to bring about pulmonary tuberculosis only very much later, have gradually acquired the characters of the human type.

Although no one has ever succeeded in experimentally producing any such adaptation, the facts to be brought out in the rest of this chapter will furnish a number of arguments in support of this hypothesis. However, it is only fair to recognize, along with R. Koch,[16] that the importance of this matter is purely theoretical, since even if it be granted that it is possible to transform the bovine into the human type by cultural means or by passages through various animal bodies, the only question which interests us practically is that of knowing in what measure the manipulation or ingestion of meat, milk or butter derived from tuberculous cattle is dangerous for man.

Now, with this special point in view, the fact should be noted that *tuberculous infection is very common in countries where bovine tuberculosis does not exist and where children are never fed with cow's milk.*

[14] Centralbl. f. Bakt., 1912, **65**, 42.

[15] Tuberk.-Arb. a. d. k. Gsndhtsamte, 1912, H. 11, 1.

[16] Internat. Congr. on Tuberculosis, 6th. Wash., 1908.

The countries where this question has been best studied are Japan, Indo-China, India, the Island of Madagascar, Turkey and Greenland.

In Japan, for example, according to Shishido, Kanda, Kitasato, Tada, and others, native children have always been brought up at the breast, often until the third year. Only recently, since the opening of certain ports to international commerce, have any dairies been established, and their output is sold almost exclusively to the foreign residents. The price of this milk is too high for the natives. When the mother is unable to nurse—which occurs rarely—the child is fed with soups or entrusted to a wet-nurse.

Tuberculosis is nevertheless as widespread in this country as in the less favored parts of Europe as may be seen from the following figures (Bruno Heymann):[17]

Mortality from phthisis per million inhabitants

Japan, from 1891 to 1895	1354
England, from 1894 to 1897	1358
Italy, from 1895 to 1897	1871 (tuberculosis of lungs and other organs)
Germany, from 1894 to 1897	2245
France, from 1894 to 1897	3023 (tuberculosis of lungs and other organs)
Austria, from 1895 to 1896	3625 (tuberculosis of lungs and other organs)

In infants under one year the death rate per 1000 is 1.3 in Japan, as against 2.3 in Germany. This mortality is particularly high at Tokio and in the large centers of population where families still live under very poor hygienic conditions despite the great progress more recently made.

In Turkey also it is only very exceptionally that an infant is not nursed by its mother or, failing her, by a wet-nurse; and yet tuberculosis is very common in all its forms and at all ages. Cow's milk has nothing to do with the propagation of the disease since the people, at least in the cities, consume but very little and that rarely as fresh milk.

[17] Ztschr. f. Hyg., 1904, **48**, 45.

In Greenland phthisis is so general that, according to C. Lange, more than a third of the deaths are attributable to it. Milk is never used, not even that of reindeer, these animals not having been domesticated as in Lapland. Man to man contagion is therefore the only form entering in. The same applies to Kamtchatka, the Polynesian islands of Oceania, Madagascar, China and Indo-China, so that the geographic distribution of human tuberculosis is not appreciably influenced by the geographic distribution of bovine tuberculosis (*see Chapters XXIII and XL*).

But unquestionably, in the different countries of Europe especially, and also in the United States and Canada, bovine tuberculosis represents a factor in the infection of the human race of such importance as to render its eradication a necessity. In Germany, for example, there die each year from tuberculosis, according to Bendix, 27,200 nursing infants. If we accept with Orth[18] that 10 per cent or 2720 of these infants have been infected with bovine tuberculosis, we must indeed recognize that the figure is not a negligible one.

A. TUBERCULOUS MEATS

Since the experiments of Chauveau,[19] Villemin, Parrot, Klebs, Toussaint, Baumgarten, Viseur, Nocard, Straus, and others, the fact has been well known that a variety of animals (cattle, swine, dogs, cats, wild animals, etc.), may contract tuberculosis if fed with meat from tuberculous animals. In man, however, since ordinarily only meat which has been previously cooked is eaten the danger is very slight.

There is no doubt that the various organs used as food, and also the muscle tissue, may contain tubercle bacilli and now and then in abundance, not only when there exist foci of softening or multiple areas of lobular broncho-pneumonia, but even when the lymphatic glands appear macroscopically healthy. The investigations of Bongert[20] and Nieberle,[21] as well as those of Haentle,[22] and later those of M. Muller and T. Ishiwara[23] carried out at the abattoir in

[18] *Drei Vorträge über Tuberkulose*, Berl., 1913, Hirschwald.
[19] Bull. Acad. méd., 1868, **33**, 1007.
[20] Arch. f. Hyg., 1909, **69**, 263.
[21] Ztschr. f. Fleisch.-u. Milchhyg., 1910/11, **21**, 237; 339; 1911/12, **22**, 12; 266.
[22] Centralbl. f. Bakt., 1914. **74**, 91.
[23] Ibid., 1914, **74**, 393.

Munich, are very illuminating in this respect. Moreover one can only approve of the measures for the total or partial seizure of meat in the abattoirs, when the animals harbor generalized or local lesions.

"In acute miliary tuberculosis," says Bongert, "and also where the evidences of a recent blood infection exist only in the viscera (and not in the meat), and finally where there are alterations of the muscular tissue and pronounced emaciation, the whole body of the animal should be considered dangerous to health and condemned."

Yet, as a result of their experiments carried out at the KK. Gesundheitsamt with material taken from various animals (bullocks, calves, swine) at the abattoir at Berlin, C. Titze, H. Thieringer and E. Jahn[24] assert on the one hand that, in local tuberculosis the blood contains no bacilli and, on the other hand, that old encapsulated tuberculous foci, which are not accompanied by recently disseminated lesions, do not in any way justify radical measures of seizure (provided the diseased tissue itself be rejected).

But Muller[25] has called attention to the fact that, very frequently, tubercle bacilli are found in the spleen and liver of cattle which, on being slaughtered, show no apparent lesions of these organs. Hans Mittel[26] insists on this extremely important fact. At the abattoir in Munich he was able to study this point in 33 tuberculous animals in which the spleen and liver appeared perfectly healthy. In 10 of these cases he was able with pulp of the spleen, and in 8 of them with pulp of the liver, to infect guinea pigs in 36 per cent of his trials.

In studying the question of the possible virulence of macroscopically healthy muscles and glands, from the point of view of food hygiene, P. Chaussé[27] recently performed some new experiments which showed that muscle does not contain bacilli (the adductor muscles of the thigh in swine, and the adductors of the thigh and the psoas or long dorsal in cattle), whereas glands of normal appearance frequently do contain them.

In Germany, in the majority of large cities, meats seized on account of tuberculosis may be cooked in special autoclaves, at the abattoir itself, and sold at reduced prices in a low-grade meat shop

[24] Arb. a. d. k. Gsndhtsamte, 1913, **45**, 364.

[25] Centralbl. f. Bakt., 1912, **62**, 335.

[26] Ibid., 1915, **75**, 113.

[27] Ann. de l'Inst. Pasteur, 1917, **31**, 1.

called a *Freibank.* This practice does not seem to be objectionable provided the cooking is carried out under the constant supervision of the veterinary inspector.

The handling of tuberculous meat by butchers or abattoir workers and the autopsying of diseased animals by veterinarians presents a much more obvious danger. Several cases have been reported of accidental or voluntary inoculation (such as the cases of Klemperer[28] in Germany and of Garnault in 1901 in France). It is however only rarely that the consequences are serious, and this is an argument tending to demonstrate the usually low virulence of the bovine bacillus for adult man.

R. Pfeiffer, Tscherning, Ravenel, Ostertag, Johne, Kurt Muller, Salmon, Spronck and Hoffnagel,[29] C. Damman and L. Rabinowitsch[30] and several other observers have described cases of accidental infection of veterinarians, butchers or workers in abattoirs. L. Mayer[31] collected 20 such observations from the medical literature up to 1906, and several others have been published since then. Most of them bespeak the low virulence of the bovine bacillus for man. Except in a few exceptional cases (those of Hartzell, of Salmon, de Troje, cited by L. Meyer), there resulted only a local infection or one limited to the gland group near the point of inoculation. The reason for this lies perhaps in the fact, as E. A. Lindemann[32] thinks, that the bovine bacillus in the human skin finds itself exposed to attenuating influences. At the same time it cannot be definitely asserted that the individuals thus accidentally or voluntarily infected did not possess a certain degree of immunity conferred upon them by a previous infection which remained benign or occult.

B. THE QUESTION OF MILK

It was shown long ago (Gerlach, 1860, Klebs 1873, Nocard, Galtier, Hirschberger, A. Ostermann[33] and others), that milk from cows with mammary gland tuberculosis may be extremely rich in bacilli (as many as 100,000 per cubic centimeter) and that this milk when

[28] Ztschr. f. klin. Med., 1905, **56**, 241.
[29] Semaine méd., 1902, **22**, 241.
[30] Ztschr. f. Tuberk., 1908, **12**, 441.
[31] Ztschr. f. Thiermed., 1906, **10**, 161.
[32] Berl. klin. Wchnschr., 1912, **49**, 1185.
[33] Ztschr. f. Hyg., 1908, **60**, 375; 410.

ingested is highly infectious, not only for calves but also for the majority of mammals like the cat (Viseur), the dog, and the monkey (Nocard, Gratia).

Statistics compiled in France by Martel, and in Germany by Ostertag, indicate that tuberculosis of the mammary gland is fairly frequent, being present in 2 to 4 per cent on the average, of animals reacting to tuberculin. In the whole of the German Empire in 1888 and 1889, the percentage of cows which on being slaughtered were found to have infection in the udder was 1.62. In Pennsylvania in the United States, Pearson found 8.75 per cent among 120 tuberculous cows. In France, in the central departments and in those of the southeast, there were 44 cases among 675 tuberculous cows, or 6.5 per cent. At Marseilles, Huon reported 21 among 698.

It is not only the cows with mammary gland lesions which constitute a grave danger, since their number is relatively small, but the experiments of Rabinowitsch and Kempner,[34] those of Adami and Martin, of Gehrmann and Evans, of Mohler, of Moussu,[35] have shown that even the milk of animals which have no clinically demonstrable mammary lesions and which simply react to tuberculin may now and then and *intermittently*, contain bacilli (*see Chapter XXXIII, D*). The question therefore arose as to the wisdom of forbidding the use as food of all raw milk from dairies where cows reacting positively to tuberculin were not rigorously eliminated.

This serious question could not be answered without the widest possible investigation in order to determine the proportion of bovine tuberculosis in children.

Now, we saw at the beginning of this chapter that this proportion although relatively small is nevertheless not a negligible quantity, since for children under 5 years the figure is 6 to 10 per cent according to W. Park, and appears still higher in certain countries such as England and Ireland (Sh. Delépine),[36] where raw milk is used very extensively.

One would expect it to be even much higher considering with what frequency authentic tubercle bacilli are present in commercial milk. At Manchester, for example, among samples obtained

[34] Ztschr. f. Thiermed., 1904, **8**, 202.

[35] Compt. rend. Soc. de biol., 1904, **56**, 617.

[36] Congr. of Royal Institute of Public Health, Paris, 1913.

in the public markets, the proportion of those containing bacilli has varied since 1898 from 5.5 to 17.6 per cent according to the year (Sh. Delépine).

In 1897 at Liverpool, of 144 samples, only 2.8 per cent were infected. The percentage indeed varies greatly, as the following figures show:

	per cent
London (MacFadyean)	22.0
Edinburgh (Philip Mitchell)	20.0
Sheffield	10.4
Birmingham	7.3
New-York (1910) (Bureau of Animal Industry)	16.0
Washington	7.0
Chicago (1910) (Tonnens)	10.5
Berlin (Pétri, Beck, Rabinowitsch)	14.0 to 30.0
Leipzig (1908)	10.5
Lauterthal-in-Harz	2.53
Milan	2.00

At Berne, Thöni[37] found bacilli in 17 of 212 specimens, or 8 per cent. Of these 212 specimens, 155 came from small individual producers and 57 from dairies where milks from several farms were mixed. In the first group of 155 there were 9 infected specimens, or 5.8 per cent, and in the second group 8, or 14.03 per cent.

If one compares the often enormous number of samples of commercial milk found infected with tubercle bacilli in the large cities with the number of cases of human infection which are certainly or probably of bovine origin, one is forced to recognize that the consumption of these milks is far from being as dangerous as might be feared. Investigations made from 1905 to 1909 in Germany by the Imperial Sanitary Office and which were published by A. Weber,[38] throw much light on this point. They pertain to 619 persons, 284 of whom were children and 335 adults. Now, among them all, 151 children and 200 adults had regularly consumed, for longer or shorter periods, raw milk from cows shown to have tuberculous lesions of the udder. Only two individuals, and they were very young and belonging to two different families, were found to have tuberculous cervical glands of a benign form. In both of these cases, the offending cow had a very severe mastitis involving the whole

[37] Centralbl. f. Bakt., 1914, **74**, 11.
[38] Tuberk.-Arb. a. d. k. Gsndhtsamte, 1906 to 1910.

gland; the other individuals who had drunk the same milk, equally raw, among whom were 8 children from 3 to 8 years, were in perfectly good health. A. Weber was forced to conclude that—and the opinion has recently been supported by Flügge and Ostermann—if infection is to be produced there must be a frequently repeated intake of a considerable quantity of bovine bacilli, a condition which is scarcely realized for the market product which as a rule is a mixture of milks from a large number of cows.

In New York, Alfred F. Hess[39] made a similar investigation. He set out first to determine the proportion of bacillus-containing milks sold by the dealers and, among 107 samples taken, found 19 (17 per cent), of which 15 came from the same farm.

Ten of the dealers selling infected milk had a total of 18 children of their own who were in the habit of drinking raw milk from the cows at home: 9 were under 2 years, 8 from 2 to 5 years, and one child over 5 years. These children were all followed and carefully examined for one year, then tested with tuberculin (ophthalmic reaction). Four of them reacted positively, but showed no clinical sign of tuberculosis, with the exception of one little girl of 2 years who had been drinking about a pint a day of the worst infected milk. She had a cervical adenitis which had to be incised at a dispensary. No member of her family was suspected of having tuberculosis.

Beitzke (of Lausanne)[40] reported a child of 14 years who at autopsy presented, in addition to caseated mesenteric and retroperitoneal gland lesions, a peritoneal tuberculosis resembling the perlsucht of cattle, and at the same time a tuberculosis of the spleen, ulcerations of the intestine and caseous tubercles in the lung. The products on inoculation were found to be very virulent for the rabbit and guinea pig. There was strong reason to believe therefore that the infection was of bovine origin, and an investigation of the family tended to confirm it. The child, whose parents and 8 brothers and sisters were perfectly well, was in the habit of drinking one or two glasses of raw milk each day at a neighboring farm. Unfortunately the information to be had at the latter was of no use, since the cows had been sold.

A. F. Hess collected observations of 44 cases of bovine infection

[39] J. Am. Med. Assn., 1911, **66**, 1322;—Studies from Research Lab., Dept. of Health, N. Y., 1908/09.

[40] Rev. suisse de méd., 1914, April 4.

of the mesenteric glands, 41 of them being in children. One of these cases, published in the Medical Report of the County of Aberdeen, had been sent to him by J. M. Adams and is worth recalling in some detail:

A farm-laborer, who had 3 daughters aged 9, 6, and 4 years, lost the oldest on the 7th of January, 1907, of meningitis. On the 18th of March following, the youngest died. Autopsy showed tuberculous lesions of the meninges and mesenteric glands, and bacilli were present in the spinal fluid. No tuberculosis was obvious in either the father or the mother. The milk drunk by these children was from a single cow which had a tuberculosis of the mammary gland. She was killed and found to have generalized tuberculosis. Cultures made by J. Milner Adams at the University of Aberdeen, from the cerebrospinal fluid of the child and from the milk and mesenteric glands of the cow, all gave characteristic bovine type bacilli (culture and virulence for the rabbit).

This case, well studied, indicates better than all the preceding, that although there is need to combat in a certain measure the exaggerated fear of the spread of bovine tuberculosis to man through the bacilli frequently contained in cow's milk, *the repeated ingestion of raw milk containing many bacilli should be regarded as dangerous, particularly for young children.*

All measures must be approved therefore which aim at preventing the supply for food purposes—especially to maternities, nurseries, schools and public institutions of all sorts—of any but pasteurized, boiled or sterilized milks, or raw milks from stables in which all the cows are periodically tested with tuberculin and kept under administrative supervision.

Milk products, such as cream, butter and cheeses, should come under the same rulings. It has been proved in fact by the researches of Schroeder and Cotton[41] of the Bureau of Animal Industry at Washington, by those of Herr and Beninde,[42] of Bang, of Roth, of A. Ostermann and other investigators, that tubercle bacilli contained in milk adhere to the fat droplets and are particularly prone to collect in the cream, where they may be recovered in large number (at times as many as 100 bacilli per gram of cream).

At the same time, in the annual report of 1907, the Secretary of

[41] U. S. Dept. Agric., Bur. Anim. Indust., Circ. No. 27, 1908.
[42] Ztschr. f. Hyg., 1901, **38**, 152.

Agriculture of the United States asserts that examination of sediment from separators in creameries through the country at large shows the presence of tubercle bacilli in 25 per cent of samples.

Broers[43] has observed that the bacilli remain alive and virulent in butter for three weeks, and F. C. Harrison[44] found that their vitality is not lost in Emmenthal cheese made in Switzerland, until between the thirty-second and the fortieth days.

Among 50 cheeses made from milk of tuberculous cows, Kankaanpaa,[45] found tubercle bacilli 7 times. Among cheeses bought in the open market, the oldest which contained tubercle bacilli had been made 200 days before.

By infecting cheeses artificially, the virulence of bacilli contained therein has been shown to diminish with time. As a matter of fact, at the end of 50 days guinea pigs could still be given tuberculosis by injecting the infected cheese, whereas after 68 days it was no longer possible. Nevertheless, the duration of virulence as well as of survival seem to vary with different varieties of cheese.

Morgenroth states that even margarine is not without its tubercle bacilli, since it is prepared in large part from the mesenteric and mediastinal fats heated only to 45°C. and made up afterwards in milk. The indication therefore is that only the suet of animals free from tuberculosis should be utilized in its manufacture.

[43] Ztschr. f. Tuberk., 1906, **10**, 260.

[44] Ann. agricole de la Suisse, 1902.

[45] Centralbl. f. Bakt., Ref., 1913, **56**, 202.

CHAPTER XXVI

SPONTANEOUS TUBERCULOUS INFECTION IN VARIOUS MAMMALS OTHER THAN MAN AND CATTLE

The mammals in which tuberculous infection is by far the widest spread, after *man* and *cattle*, are *swine* and *dogs*.

Other domestic animals, the *horse*, *ass*, *goat*, *sheep*, *cat*, *rabbit*, *guinea pig*, and *rat*, may contract it when they live as cohabitants for long periods with *tuberculous cattle or man*, but are little susceptible to this infection.

A. SPONTANEOUS TUBERCULOUS INFECTION IN WILD ANIMALS

Wild animals such as *monkeys*, the *large felines* (*lions and tigers*), *antelopes*, *elephants*, etc., never contract tuberculosis *spontaneously;* but, *in captivity*, they are susceptible to it.

Contagion takes place exclusively under conditions of domestic life and in the social groupings. For this reason buffaloes and wild boars, for example, remain entirely exempt, just as do the monkeys in the tropical forests, although it is a matter of common knowledge how easily the latter become tuberculous when in menageries.

All varieties of *monkey* can be easily infected with bacilli of either human or bovine origin. The anthropoids (*chimpanzees*, *orangs*, *gibbons*) are extremely susceptible (Dungern).[1] The macaques (*Macacus sinicus*, *M. rhesus*, *M. cynomolgus*) are rather more so than the *Semnopithecinae* and the *Cercopithecidae* (*Plate XIX*).

In the Berlin zoölogical garden, L. Rabinowitsch[2] was able to study 45 monkeys that died of tuberculosis and that had been infected either in cages where they lived alone or in common cages where they were exposed to infection from outside as well as to contagion among themselves. Among these monkeys of various species only 5 had pulmonary tuberculosis; 9 had tuberculosis of the glands and abdominal viscera; 31 had both abdominal and pul-

[1] München. med. Wchnschr., 1906, **53**, 4.

[2] Deutsch. med. Wchnschr., 1906, **32**, 866.

monary tuberculosis. By means of culture and inoculation of rabbits, 19 of 27 animals examined were found to be infected with human type bacilli and 3 with bacilli of the bovine type.

In 5 other monkeys, Lindemann[3] isolated a culture of the bovine type in 3 instances and of the human type in 2 instances. In the 2 monkeys presenting the human type, the disease was generalized in all the viscera.

E. F. Southard[4] (of Boston) chanced to run across a tuberculosis of the lumbar spine (Pott's disease) in a half-grown *Macacus cynomolgus* which was a pet in an American family.

From Nocard's[5] experiments, it seems that monkeys are more susceptible to bovine than to human tuberculosis when they are infected by the method of ingestion with equal weights of culture of each of the two types. Schweinitz and Schroeder, Ravenel, de Jongh, Koch and Schuetz, Imbach, Cipollina, and Gratia,[6] arrive at the same conclusion.

The lower monkeys react to tuberculin inoculated subcutaneously but are less sensitive to it than man and, in them, the local reactions (*ophthalmic, intradermic or cutaneous*) are generally not at all marked. In the chimpanzees, on the contrary, local reactions are very pronounced (Burnet).[7]

At the Berlin zoological garden, among 39 monkeys tested by the cuti-reaction of Pirquet by H. Ziemann, only one reacted in a clear-cut manner: this was a very sociable gibbon which had frequent and friendly relations with the public and which died about 20 months later. A *cynomolgus* also reacted weakly (*ophthalmic* and *cuti*).

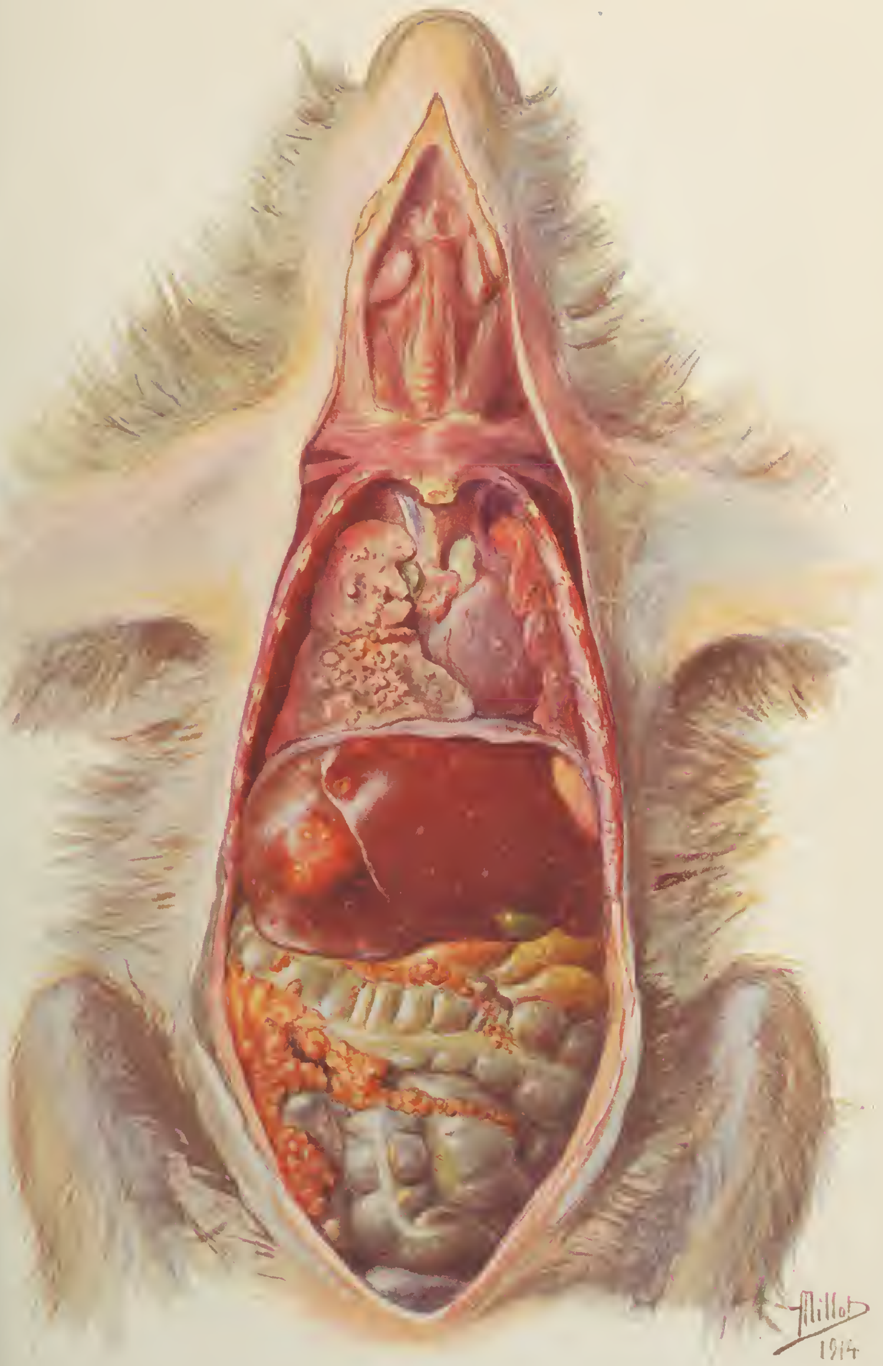
In menageries it is not rare to observe cases of tuberculosis among the large felines which are usually fed with offal from the abattoirs. I. Straus in 1894[8] reported the case of a 5 year old *lioness* which died of phthisis in a menagerie where she had been for 3 years. Autopsy revealed the existence of pulmonary tuberculosis, with no lesions visible in the other organs. The lungs were full of very

[3] Deutsch. med. Wchnschr., 1912, **38**, 1921.
[4] J. Med. Research, 1906, **14**, 393.
[5] Rev. gén. de méd. vétér., 1903, i, 1.
[6] Internat. Congr. of Hyg. and Dem., 13th., Brussels, 1903.
[7] Compt. rend. Soc. de biol., 1912, **73**, 248.
[8] Arch. de méd. expér., 1894, **6**, 645.

PLATE XIX

Spontaneous generalized tuberculosis, of digestive origin, in a monkey from the "Jardin des Plantes" by courtesy of MM. Trouessart and Anthony of the Museum of Natural History.)

Lesions of miliary tuberculosis of the lungs, pericardium, liver, spleen, omentum and mesentery.


Millot
1914

small cavities, with areas of hepatization which contained abundant muco-pus rich in bacilli.

Jensen[9] too had the opportunity of autopsying several animals from the zoological garden at Copenhagen. He found tuberculous lesions, confirmed by bacteriological examination, in an *arctic fox* (*Canis lagopus*), in one *horse*, in six *bears* dead of phthisis with cavities, in two *lions*, in one *royal tiger*, in a *black panther* and in a *jaguar*.

Bergeon[10] likewise autopsied at Saigon a female panther of 3 years that had lived 16 months in captivity in the zoological garden. She presented extensive lesions in the liver and both pleurae, which were thickened, adherent, and full of serous fluid. In the right lung were a large number of miliary tubercles and caseous chalky foci. The bronchial and mediastinal lymph nodes were very large and contained cheesy pus. Bergeon tells us that similar findings have been disclosed from time to time in menagerie animals in Indo-China, and he adds that at Saigon they are fed with poor quality meat.

Dammann and Stedefeder[11] had occasion to autopsy a young elephant which had tuberculous lesions in the lungs and in some of the vertebral bodies. Guinea pig and rabbit inoculation as well as culture on glycerin broth indicated that the bacillus isolated was of the human type. Inasmuch as the elephant had been very little in association with other animals, the authors thought that he had infected himself through food, particularly by pieces of bread fed to him by the public and which might well have been contaminated with tuberculous sputum.

From still another case of exclusively pulmonary tuberculosis in an elephant, H. Thieringer[12] also isolated a bacillus of the *human* type.

At the Museum of Natural History in Paris, Lucet[13] found in a *llama* a double caseous pneumonia with some very small sparse tubercles in the liver, and A. Schultze[14] in Berlin saw intestinal tuberculosis in a *roe* bottle-fed with raw cow's milk.

[9] Deutsch. Ztschr. f. Thiermed., 1891, **17**, 295.
[10] Rev. vétér., 1909, Feb. 1.
[11] Deutsch. tierarztl. Wchnschr., 1909, **17**, 345.
[12] Berl. tierarztl. Wchnschr., 1911, **27**, 234.
[13] Soc. de méd. vétér. pratique, 1909, July 7.
[14] Berl. tierarztl. Wchnschr., 1911, **27**, May 11.

McCoy and Ch. W. Chapin[15] reported the existence of tuberculosis in the *ground squirrels* (*Citellus beecheyi Richardson*) which are found in great number about San Francisco. Among 225 of these animals, which they were examining with a view to studying their rôle in the propagation of plague, they found 5 with tuberculous lesions of the glands, viscera, and lungs, and from them they isolated bacilli of the *bovine* type. Infection had probably taken place through the dejections of cattle scattered over the country side.

Spontaneous tuberculous infection occurs very rarely in *tame rabbits* and very rarely in the raising of *guinea pigs*, although these animals are extremely susceptible to artificial infection. It is observed however, though exceptionally, in laboratories, and is then brought about by accidental contamination of the food or bedding. Rothe,[16] at the Sanatorium Heidehaus (Hanover), in 1909, had occasion to study such an epidemic occurring in a rabbit farm. Infection was found to be of bovine type.

A. Weber and Bofinger studied a case of spontaneous tuberculosis in a *grey mouse;* the bacillus proved to be of the *avian* type. *White mice* are very easily infected experimentally with this virus (A. Koch and L. Rabinowitsch,[17] Strauss, Römer[18]). Intraperitoneal injection of doses of culture, even though large (1 centigram to 1 mgm.), produces in these small rodents a disease which, as Koch[19] had observed, always takes a chronic form with an extraordinary multiplication of bacilli in all the organs. Contrarily, inoculation by the same peritoneal route, of 1 mgm. of a culture of bovine bacilli, leads often enough to early death, within a few days, through bacillemia.

From the experiments of R. Trommsdorf[20] *intravenous* inoculation into the mouse should enable one to differentiate bovine and human tubercle bacilli: those of human type, in a dose of 1 mgm., are said not to produce any macroscopically visible lesions by the end of the 4th week, whereas the same dose, or even 0.1 mgm. of bovine bacilli, is said to cause a general infection with pulmonary

[15] J. Med. Research, 1911, **25**, 189.
[16] Veröffentl. d. R. Koch Stift. z. Bekämpf. d. Tuberk., 1913, H. 4, 1.
[17] Virchow's Arch., 1907, **190**, B., 246.
[18] Beitr. z. exper. Therap., 1903, H. 6, 1.
[19] Mitteil. a. d. k. Gsndhtsamte, 1884, **2**, 66.
[20] Arb. a. d. k. Gsndhtsamte, 1909, **32**, 568.

localizations very apparent after the same period and visible as early as the fifteenth day.

B. TUBERCULOSIS OF DOMESTICATED RUMINANTS OTHER THAN CATTLE

E. Manson recognized the existence, although very rare, of tuberculosis in *camels* in Egypt. The bacillus isolated had the characters of the *bovine* type.

In domesticated animals *confined permanently or temporarily in stables,* spontaneous tuberculous infection is infinitely more common. At the same time it is relatively rare in *sheep* and in *goats.* They can scarcely be infected except when they live together with *cows,* which in general is rather exceptional, since sheep and goats live in flocks in the open air or in folds. Yet it is reported now and then in the statistics of the abattoirs. In Germany, according to Karl Hertha,[21] 0.77 per cent of goats killed for meat are found tuberculous. The infection, due exclusively to the bovine bacillus (the human bacillus is only very slightly virulent for the goat), assumes the pulmonary form most frequently, although there have been reported cases of generalized tuberculosis and fairly numerous observations of mammary tuberculosis (Delmer, Bulling, Rabieaux, Schroeder).

During the first eight months of the year 1911, among 2843 goats slaughtered at the abattoir of Saint-Etienne, 5, or 0.17 per cent were found infected with tuberculosis. Four of them had generalized lesions with infection of one or several retro-mammary glands and, consequently, were capable of giving infectious milk (G. Morel).[22]

The use of goat's milk, in the raw state, may therefore present some danger.

C. TUBERCULOSIS IN THE HORSE AND IN THE ASS

Tuberculosis is likewise infrequent in the *horse.* In our abattoirs it is found barely once in 15,000 animals, and it is still more rare in the case of the *ass* (Césari).[23] It manifests itself by emaciation, loss of appetite, extraordinary polyuria, an increasing lack of aptitude for work, a practically constant rise of temperature from 1 to

[21] Arb. a. d. hyg. Inst. d. k. tierarztl. Hochschule, Berl., 1910, No. 6.
[22] Hyg. de la viande [etc.], 1911, **5**, 642.
[23] Ibid., 1910, **4**, 333.

PLATE XX

1. Pulmonary tuberculosis in the horse (sarcomatous form). Anatomical specimen from the abattoir at Lille.

2. Tuberculosis of the spleen in the horse. (Anatomical Museum of the Veterinary School at Alfort.)

I

II

1.5°C., with irregular periods of still higher fever. Pulmonary and visceral forms are the most common, although there are observed at times pleural or peritoneal forms with large effusions into the serous cavities. Different types of bacilli have been isolated, but most frequently it is the *bovine* (Zwick and Zeller),[24] with the *human* type now and then. Cases of equine abdominal tuberculosis of avian type have also been reported (Nocard).[25] It appears then that the horse, although rarely infected spontaneously with tuberculosis, is susceptible to all forms of the virus of warm-blooded animals.

Davis told of a small epidemic among four horses, occurring during a period of 3 years, on a farm where there were never more than 6 horses at a time. A young mare raised on cow's milk died of tuberculosis; her dam was tuberculous and also the mother of the dam. The dam died soon after the young mare was born, and showed multiple lesions; one ovary in particular was almost entirely destroyed.

Pulmonary localizations in the horse fairly often take the form of an infiltration of sarcomatous appearance, manifesting itself in the form of tumors of varying size, or involving the whole of the lobe (*see Plate XX*). The tumors are made up of a conglomeration of tuberculous follicles with no inflammatory reaction round about and with no caseous matter in the center.

When the spleen takes part in the same process it is found full of large rounded white masses, from the size of a hazel-nut to that of an apple, and non-caseous.

The liver too many be the seat of similar tumors. In the intestine there are found at times thick soft polyp-like excrescences, or again more or less extensive ulcerations, particularly in Peyer's patches.

The rarity of tuberculosis in horses makes it difficult to obtain authentic information as to the manner of infection. It seems to be most often by means of cow's milk fed to colts with a therapeutic end in view or to prepare them for sale. Such at least is the opinion of MacFadyean[26] and of Bang. Dopheide[27] (of Steinfurth) reported a case of transmission of tuberculosis from a woman to a horse.

[24] Arb. a. d. k. Gsndhtsamte, 1913, **42**, 483.
[25] Bull. de la Soc. méd. vétér., 1896, p. 248.
[26] J. Comp. Path. and Therap., 1891, **4**, 383.
[27] Arch. f. Thierheilk., 1900, **26**, 353.

D. TUBERCULOSIS OF SWINE

Infection of *swine* is much more frequent, but is observed solely in countries where these animals are carelessly fed with refuse from dairies. At the meat packing abattoirs in Chicago, among 100 swine an average of 3 were found with some tuberculous lesion, generally not very extensive however and most often in the glands. (Of 100 tuberculous swine, 50 had lesions in the pharynx, 35 in the peribronchial glands, and 15 had generalized tuberculosis.)

The proportion is lower in animals coming from the far West where swine are fed entirely on vegetable tubers or cooked grains. The same applies to Europe. In Denmark where swine tuberculosis used to be common, the disease has completely disappeared wherever the animals are fed only with pasteurized whey.

In the Argentine Republic, where bovine tuberculosis is very rare, there has been noted during the last few years a considerable extension of swine tuberculosis. The latter is therefore of human origin or, more probably, of avian origin. At the abattoir of Buenos Aires in 1905, among 48,077 swine slaughtered, 4319 were found tuberculous or 8.98 per cent. Of this number, 630 had generalized tuberculosis and 3689 had lesions localized chiefly in the glands of the neck, the pharyngeal and sublingual glands.

According to P. de la Cruz Mendoza,[28] who reports these figures, the diagnosis of tuberculosis in the swine cadaver requires a minute examination of the lymphatic system. Only numerous sections enable one to find either caseous foci or small tubercles in the intermuscular glands.

If the tuberculosis is generalized, the fat is allowed to be used only after sterilization with heat under pressure. If the lesions have spread to the various viscera, the meat is cut into pieces of fixed size, sterilized and delivered to the Assistance Publique (Department of Charities of Paris) with a tag of control. Sale is permitted only if the localized lesions are fibrous or calcified.

Since the life of swine is, in general, brief, it will be understood why the forms of tuberculosis ordinarily encountered in these animals are chiefly glandular and abdominal. It often happens that the glands of the neck are first involved, this localization resulting probably from the fact that the animal infects itself digging with its

[28] Boletin de Agricultura y Ganaderia, January, 1906, p. 34.

snout into cattle manure or household slops contaminated with bacillus-containing sputum. When the infection is of some age, it invades other gland groups, those of the pleura, of the lungs or other viscera.

Sometimes one finds the lungs full of gray miliary granulations, translucent or yellowish and opaque, or else studded with large tubercles with fibrous walls and caseous centers, of the size of a pea or hazel-nut, or again one finds areas of caseous pneumonia (*see Plate XXI*).

The liver and particularly the spleen may also be the seat of rounded nodes which at times have a sarcomatous appearance, or again are softened at the center.

Tuberculous infection of swine has been reported not infrequently following castration. This infection is at times of the human type—it results then from contamination of the wound by the saliva or sputum of the operator,—at times of the bovine type following the deposit of milk or bovine excrement upon the operative wound. Chaussé[29] has published two interesting observations of this sort. In both cases the inguinal, iliac and sub-lumbar glands were the first to be involved.

In a recent very detailed work,[30] this same scientist showed that swine become infected most often by way of the tonsils and cervical lymphatics. The lungs are said not to be involved until the infection becomes generalized. In France the proportion of swine found tuberculous in the abattoirs rarely exceeds 0.25 per cent.

Cases have been reported in swine of tuberculosis of the eye, of the ear, of the nervous centres, genital organs, bones, joints, and also acute generalized forms with nodules or crops of tubercles even in the interstices of the muscular fibers.

The bacilli isolated from tuberculous lesions of swine usually belong to the *bovine* type. But these animals are susceptible, both spontaneously and experimentally, to bacilli of the *avian* and *human* types (G. Dean and Todd). Among 59 cases studied by the English Royal Commission, the bovine type was found 50 times, the human type 3 times, avian type 5 times, mixed bovine and avian types in one instance.

A. Eastwood and F. Griffith[31] made, in England, a complete

[29] Rec. de méd. vétér., 1910, **87**, 297; 645.

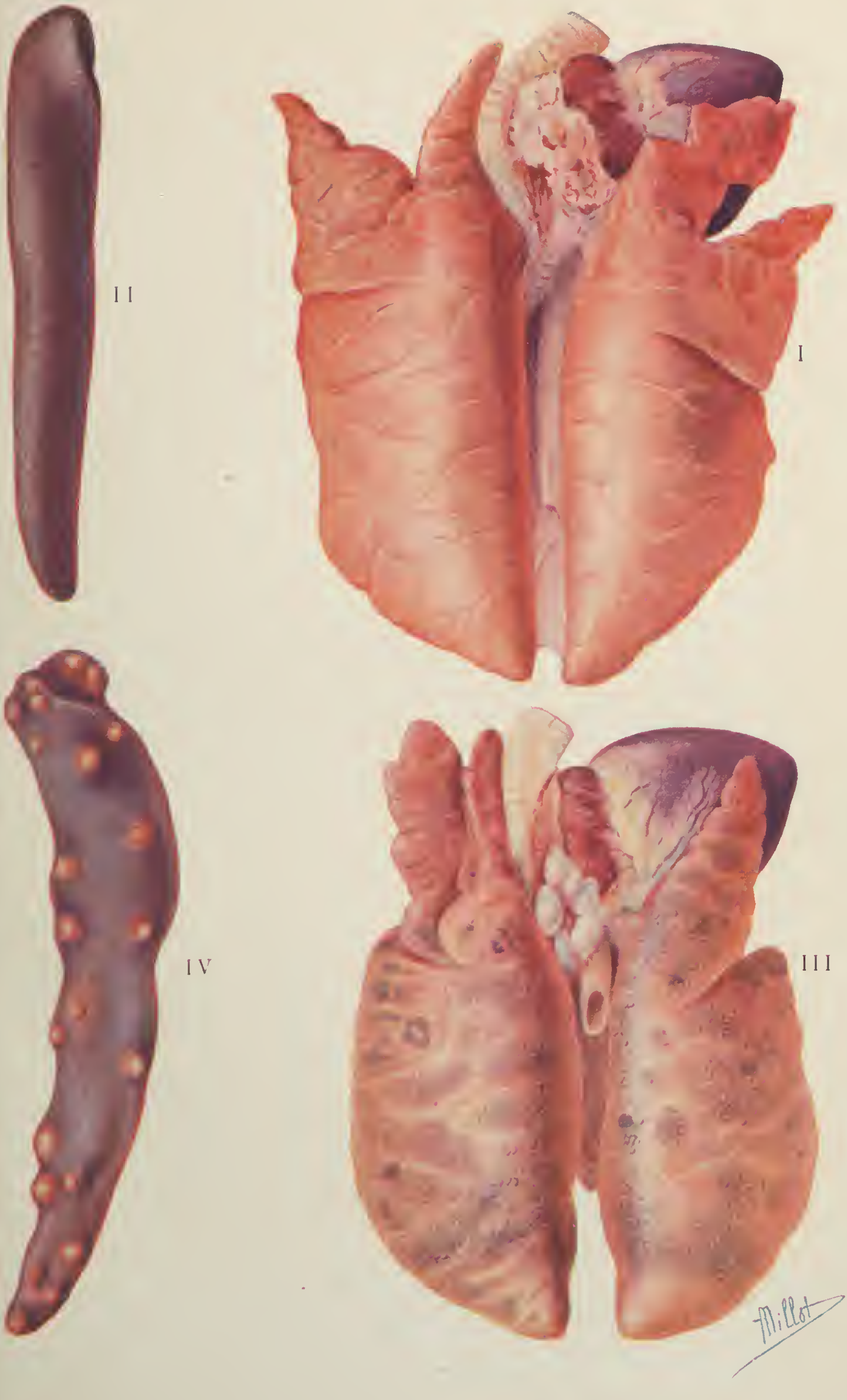
[30] Ann. de l'Inst. Pasteur, 1915, **29**, 556; 633.

[31] Rep. to the Loc. Gov. Board, No. 91, 1914.

PLATE XXI

1. The lung of a healthy swine, from the Bureau of Animal Industry at Washington.
2. Spleen of a healthy swine, from the Bureau of Animal Industry at Washington.
3. Lung of a tuberculous swine (miliary tuberculosis with caseous tracheo-bronchial glands.
4. Spleen of a tuberculous swine (abattoir at Lille).

Plate XXI.



study, both anatomically and bacteriologically, of 100 swine with lesions of localized tuberculosis. They cultivated bacilli from 78 of these animals; 26 were of the avian type, 47 of the bovine type, 1 alone of the human type, 1 a mixture of avian and bovine, 1 a mixture of bovine and human, and 2 strains were atypical.

In swine infected with avian bacilli, the disease is localized more strictly to the glands of the digestive tube and, in general, glands infected with this type are less swollen than those infected with bovine or human bacilli.

In Germany, Junack[32] also has confirmed the relative frequency of infection of swine by avian tuberculosis. This type may be suspected simply on microscopic examination which shows the bacilli present in the caseated and calcified lesions in extreme abundance, as well as a complete absence of giant cells.

Schroeder and Mohler,[33] who made an excellent study of the ordinary modes of swine contagion in the United States, whether through milk or other contaminated food, or from the dung of tuberculous cows, drew attention to the fact that by reason of the relatively small size of their lungs, the temperature in these animals is extremely irregular (average 38.9°C.), so that the thermometer should not be relied upon in judging tuberculin reactions unless each animal has been separately confined in a very quiet place for 12 hours before first taking the temperature and before the injection of tuberculin.

The dose of tuberculin to be used in swine varies from 0.1 cc. to 0.3 cc. of raw tuberculin, according to the age of the animal.

It is generally more convenient to have recourse to the *ophthalmic reaction* or the *intradermic reaction* recommended by Moussu. One drop of a 1 in 10 dilution is then inoculated into the dermis of the antero-external portion of the ear (*see Plate XVIII, 2–3*).

E. TUBERCULOSIS OF THE CAT AND DOG

Tuberculosis of *cats* and that of *dogs* has been the object of careful study by many workers, chiefly Viseur,[34] Cadiot,[35] Douville,[36]

[32] Ztschr. f. Fleisch. u. Milchhyg., 1914/15, **25**, 17.

[33] Reports of the U. S. Dep. of Agriculture, Washington, 1906.

[34] Rec. de méd. vétér., 1875.

[35] Ibid., 1891, pp. 108; 250; 587:—Rev. scient., 1914, 385.

[36] Bull. Soc. centr. de méd. vétér., 1910, p. 257.

F. J. Taylor, Jensen, Eber, Galli-Valerio, Lender and Petit (of Alfort), and Chaussé.[37]

Petit and Basset[38] published an account of 32 autopsies of phthisical dogs performed during one year at the Alfort school, where the proportion of tuberculous dogs presented for examination averages 1 in 225 according to Cadiot. In Germany, Eber, Froehner, and Muller put this proportion at 2.75 per 1000.

It seems that in France, for some years, canine tuberculosis has been growing more and more frequent. Petit states that, from 1900 to 1904, it increased from 4.57 to 9.11 per cent of the animals which he autopsied. But Douville,[39] whose recent statistics deal with approximately 20,000 dogs, estimates that the amount of canine tuberculosis is being maintained at about 4 to 4.2 per cent. Race and age are without influence.

The same observer pointed out the relative frequency of tuberculosis in dogs which are much about cafés, public drinking places and restaurants, where they lick up infectious sputum from the floor. Of 100 tuberculous dogs, 51 belonged to proprietors of such places and 23 had been in prolonged contact with persons who were ill.

In tuberculous dogs, the lesions are for the most part found localized in the lungs, pleura, liver and kidneys, and in the thoracic and abdominal lymph nodes. The spleen is almost always exempt. Infection usually occurs by the digestive tract. Since it is ordinarily the result of the ingestion of infectious sputa, the bacilli isolated belong in the majority of cases to the human type. In the opinion of Sticker, the dog is much more susceptible to human virus than to the bovine virus if inoculated intraperitoneally.

H. Schornagel,[40] in a study of 11 cases of canine tuberculosis, obtained 8 cultures of which only two were of the bovine type. Four were of the human type and two were transitional forms.

There appears to be no doubt then that the dog, contaminated by man, may in its turn become dangerous for the master through intimate association.

The same applies to the cat (Cadiot); the latter however is also often infected with the bovine bacillus through drinking bacillus-containing milk. In the large cities about 1 cat per 100 becomes tuberculous (Douville).

[37] Compt. rend. Soc. de biol., 1909, **66**, 1095.

[38] Rec. de méd. vétér., 1900, pp. 342; 405; 1901, pp. 5; 85; 162.

[39] Rev. gén. de méd. vétér., 1914, May 1.

[40] Inaug.-Diss., Utrecht, 1914.

CHAPTER XXVII

TUBERCULOUS INFECTION IN BIRDS

Birds, like mammals, are susceptible to tuberculous infection, and the latter—as, also in the case of mammals—occurs chiefly in species which live either domesticated or in captivity, in proximity to man or cattle. *Nowhere in the world is the disease observed among the wild species of birds.*

Through a slow adaptation to the body of these animals, warmer blooded than mammals, the tubercle bacillus has acquired in birds certain distinctive characters, both physiological and cultural. These attributes of the avian type are much more specific than those which distinguish the *human* and *bovine* types; but the specificity in this case is still not absolute.

This individuality of species escaped Robert Koch and the early bacteriologists who obtained pure cultures. In 1889 Rivolta[1] attempted to establish such a relationship and a little later Maffucci[2] demonstrated it and Robert Koch[3] declared himself convinced. "I do not hesitate," he writes, "without entering further into details of differentiation, to regard the bacillus of tuberculosis in hens as a species apart, although very closely related to the true bacillus of tuberculosis. As a result there is presented an important problem from the practical standpoint, that of knowing whether the bacillus of tuberculosis in hens is pathogenic for man. This question can be answered only when someone by repeated search shall succeed in finding this bacillus in man or in establishing, through a sufficient series of negative findings, that it does not exist in the human race."

A. PHYSIOLOGICAL CHARACTERS OF THE AVIAN TUBERCLE BACILLUS

For the first experimental research clearly showing the distinctive characters of the avian bacillus we are indebted to I. Straus and

[1] Gior. d'Anat. e. Fisiol., 1889, No. 1.

[2] Rif. medica, 1890, May:—Ztschr. f. Hyg., 1892, **11**, 445.

[3] Internat. Congr. on Med., 10th, Berl., 1890.

Gamaleia.[4] Their cultures were obtained originally from a hen's spleen which they had inoculated upon serum, plain agar, glycerin agar and sugar agar. Every culture had grown, although it is extremely difficult to obtain growths of human or bovine bacilli in the beginning on media other than serum, glycerin agar or the egg media of Dorset or Lubenau.

On glycerin serum or on agar, colonies appear at the end of the first week in the form of small rounded white spots, moist and glistening like droplets of wax. These spread and fuse together in a fatty layer, which is at first uniform, but which on aging becomes wrinkled and takes on a yellowish grey tint, remaining soft however instead of scale-like as with the human bacillus.

Transplanted upon fluid media (4 per cent glycerin broth), the avian bacillus grows abundantly at the surface. It spreads as a thin grumous film and develops also at a depth in the form of small rounded granules, the intermediate fluid remaining perfectly clear.

The odor of the cultures is much the same, although somewhat less strong and more sour than that of human bacilli and of bovine in particular.

The optimum temperature for growth of the avian bacillus is between 40° and 43°C. But it grows, although more slowly, at a temperature as low as 28°C., which is not true of the mammalian bacilli. The latter refuse to grow below 36°C.

Moreover the vitality of the avian bacillus is much greater. It remains alive for one or two years on artificial media; yet it gradually loses its virulence unless care is taken to replant it at least every two months.

From the morphological point of view the avian bacillus is exactly like the human bacillus. It occurs in cultures in the form of slender, elongated rods, often granular, straight or slightly curved. It stains very well with Ziehl and is as acid fast as the human bacillus.

O. Bang[5] finds that cultures on glycerin broth pass through the same phases as those of the bovine bacillus; acid at first, they become neutral, and afterward strongly alkaline.

All sorts of birds, whether tame or in captivity, are susceptible to avian tuberculosis. Those most commonly infected are the ordinary barn-yard hens, pigeons, pheasants, pea-fowls, guinea-

[4] Arch. de méd. expér., 1891, **3**, 457.

[5] Centralbl. f. Bakt., 1907, **43**, 34; 1908, **46**, 461.

fowls, turkeys, swans, canaries, finches and parrots. It has been observed in zoological gardens in vultures, ostriches, nandus (Mme Phisalix)[6] ibis, herons, gulls and eagles (L. Rabinowitsch).[7] P. Riegler[8] encountered it five times in crows (*Corvus vorax*). It is rather rare in ducks (E. King, Cadiot) and in geese (I. Straus). The sparrows which pick about in infected chicken yards are very susceptible to it and probably constitute one of the principal elements in the dissemination of the disease among barn-yard fowls (Van Es).[9]

French[10] called attention to the extreme susceptibility of polar birds to tuberculosis and he has made a study from this special point of view of a variety of arctic swan (*Olor colombianus*). These birds on migrating to South Carolina and being made captive quickly succumb to this infection.

B. PATHOLOGICO-ANATOMIC CHARACTERS OF TUBERCULOSIS IN BIRDS

Visceral lesions are the most common. They are located chiefly in the liver, spleen and lymph nodes of the abdominal cavity.

The liver appears enlarged and covered with plaques of a yellowish white color, rounded or irregular in form and varying in size from a head of a pin to that of a hazel-nut. Sometimes there is found only a fine white stippling made up of a multitude of opaque granulations (*see Plate XXII*).

The large tubercles are composed of masses of leucocytes or epithelioid cells surrounding a sort of tissue block in a state of granulo-fatty degeneration and teeming with tubercle bacilli.

In the spleen are often found agglomerations of tubercles forming a large yellowish white mass, which may be caseous and very friable, or fibrous and hard.

The lymph nodes of the abdominal cavity may attain a very large size, even that of an egg or apple. Their accumulation into a multilobular packet may push back and compress all the organs.

The intestine is often ulcerated and its walls infiltrated with large

[6] Bull. Museum d'Histoire naturelle, 1903, No. 7, 368.

[7] Virchow's Arch., 1907, 190, Beih., 196:—Arb. a. d. path. Inst. zu Berl., Feier. Johannes Orth, 1906, 365–436.

[8] Arch. veterinaria, 1912, No. 3.

[9] Berl. tierarztl. Wchnschr., 1914, p. 575.

[10] Amer. Vet. Rev., 1904, April.

PLATE XXII

Generalized avian tuberculosis in a hen. Large surface lesions of the liver and pulmonary infiltration (from nature).

PLATE XXIII

1. Parrot with tuberculous lesions of the crest (verrucous, scaly tubercles).
2. Foot of a goshawk (Astur maroccanus), with scaly verrucous tubercles.
3. Tuberculosis of the liver of a goose.
4. Section of a tuberculous lobe of the liver of a goose.
5. Section of a tuberculous focus in the liver of a goose (from anatomical specimens of Max Koch and Lydia Rabinowitsch).


III
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tubercles which project externally into the peritoneal cavity. The lungs, kidneys and ovaries are in general but little affected, or else they are full of small granular foci or caseous masses.

The joints commonly become the seat of chronic lesions in the serous membranes, ending in ankylosis (gout of birds).

The air sacs, pericardium and myocardium are seldom affected. On the other hand, there are observed not uncommonly tubercles or tuberculous ulcerations of the skin, chiefly about the bill, on the comb or on the feet. This form, almost always accompanied by visceral lesions, is found principally in the parrot. Grayish tumors, of a horny appearance and covered with crusts, then develop upon the eyelids, at the commissure of the bill, upon the tongue, upon the skin of the skull, on that of the wing, or over the joints. They form scaly prominences which overlie tubercles stuffed with bacilli (*see Plate XXIII*).

Froehner,[11] and later Eberlein,[12] observed a large number of tuberculous parrots at the clinic of the veterinary school in Berlin. Among 154 of these birds they found 56 cases. Cadiot and Roger were able to collect 27 cases.[13]

C. SYMPTOMATOLOGY AND PATHOGENESIS OF TUBERCULOUS INFECTION IN BIRDS

When there are no external lesions in which the bacilli are easily found, tuberculosis reveals itself in poultry or in caged birds by loss of activity, almost constant somnolence, loss of appetite and very pronounced emaciation. The pectoral muscles atrophy and the breast bone tapers off to a knife edge. A persistent diarrhea sets in early and the animal succumbs completely exhausted. In this stage the fecal matter often contains a very great number of bacilli whose dissemination contributes to the spread of the disease in poultry yards, aviaries or cages.

Birds whose feet thus become impregnated with virus contaminate themselves very readily, either by scratching at the eyes or at the commissure of the beak with their claws—a common trick—or by swallowing bacilli with their grain or other food.

Birds may be experimentally infected in several ways: intrave-

[11] Monatsh. f. prakt. Thierheilk., 1893, p. 51.

[12] Ibid., 1894, p. 248.

[13] Compt. rend. Soc. de biol., 1895, **47**, 812; 1896, **48**, 103.

nously, intraperitoneally, by the digestive tract, or even by subcutaneous or intramuscular inoculation. The incubation period varies from 2 to 12 months according to the mode of infection and the dosage of virus introduced into the body.

The digestive tract is unquestionably the principal pathway of infection. Nothing is easier than to infect hens, pigeons, all sorts of poultry or captive birds, or even birds of prey, by having them ingest either virulent fecal matter or pure cultures of avian bacilli. Weber and Bofinger[14] thus contaminated 17 out of 21 hens with a single ingestion of liquid culture. L. Rabinowitsch[15] was equally successful using bacillus-containing excrement. In the opinion of Wolffhugel,[16] *tuberculosis of birds is always of alimentary origin.*

Meanwhile it is an established fact that infection may also be transmitted hereditarily through the egg becoming contaminated with bacilli in the oviduct. Baumgarten and L. Rabinowitsch have brought experimental proof of this. And lastly, I succeeded with R. Letulle in giving to hens a fatal tuberculosis, generalized throughout the viscera of the abdominal cavity and in the lungs, by simply instilling one drop of a culture of avian bacilli into one of the eyes. The bacillus therefore, as in mammals, may enter into the body through the mucous membranes and by all the paths of lymphatic absorption.

D. VIRULENCE OF MAMMALIAN TUBERCLE BACILLI FOR BIRDS

It is a well known fact that tuberculosis of the *parrot,* ordinarily of avian type, may be brought about by a bacillus which presents all the characters of the mammalian bacillus. There have been a number of such observations reported (Cadiot, Gilbert and Roger) and experimentation leaves no doubt as to the susceptibility of the *psittaci* to the two forms of tuberculosis.

One of the observations published by Gilbert and Roger is particularly suggestive from the point of view of etiology.

It has to do with a parrakeet which, during 8 years in the same house, was always in good health. In August 1894 its owner began to cough. Four months later the bird showed tuberculous plaques on the cheeks; microscopic examination at this time revealed the

[14] Arb. a. d. k. Gsndhtsamte, 1904, H. 1, 83.

[15] Deutsch. med. Wchnschr., 1904, **30**, 1675.

[16] Monatsh. f. prakt. Thierheilk., 1904, **15**, 457.

presence of the bacillus of Koch in the cutaneous lesions of the animal and in the sputum of the master. The latter, who was very affectionate with his bird, used to chew food which the bird then took from his mouth. This parrakeet had no contact whatever, even temporary, with other birds. Its food consisted of grains, boiled milk, cafe au lait and material chewed by its master. The latter died in July 1895, that is to say at the end of one year.

In another observation by the same authors, the human origin is no less obvious. A man who developed pulmonary tuberculosis in 1887 and finally died of it in 1895, had purchased in 1890 a very beautiful parrakeet which then had no cutaneous lesions. Early in 1894, the bird, which was often kissed by its master and used to come and eat from his mouth, showed on the lower lid of the left eye a greyish nodule which gradually increased in size and ended by involving the whole of the lid.

Cadiot, Gilbert and Roger succeeded furthermore in tuberculizing three parrakeets with infectious matter of canine origin through repeated scarifications of the top of the head. At the point of inoculation the skin became thickened and warty, and little by little the lesions spread to the neck, back and feet. About the jaws a sort of membrane formed, and the eyes became almost entirely covered by vegetations which developed upon the lids. One of these parrakeets did not die until after 13 months; another after 119 days. At autopsy no visceral lesions were found.

By directly inoculating tuberculous products from several parrots, the same workers found them more virulent for the guinea pig than for the rabbit, which is further evidence of the close relationship to the human virus.

In other experiments, Cadiot, Gilbert and Roger[17] tried to infect 39 hens and one pheasant with tuberculous material from man, cattle and dogs. A suspension of virulent bacilli was injected either intravenously, or into the peritoneum or by both of these paths simultaneously. None of the birds died. Thirty-eight were sacrificed at intervals of from 11 to 252 days. In 5 only, very small tubercles were found in the peritoneum, the liver and the spleen. These lesions had been produced by the human virus since, on reinoculation into the guinea pig, they caused a generalized tuberculosis and could not be transmitted to a new hen.

[17] Compt. rend. Soc. de biol., 1891, **43**, 20; 66; 81.

I. Straus and Gamaleia were never able to tuberculize hens or pigeons with human bacilli, even by inoculating considerable doses of culture subcutaneously, into the veins, into the muscles or into the crest. On the other hand, J. Courmont and Dor,[18] and later Bang,[19] had some particularly fortunate results, while Nocard[20] fed young fowls with a paste of glands and lungs from tuberculous cows and not one of them contracted tuberculosis. They were found equally resistant to several inoculations of tuberculous material from human, bovine and porcine sources, whether into the muscles, peritoneum or veins. The outcome was the same in the experiments of Maffucci.[21] Weber, Titze and Weidanz[22] fed canaries with bovine, human and avian bacilli. The last type was the most virulent. Bovine bacilli caused death and the organs contained tubercle bacilli. Human bacilli produced no lesions. However the liver of one canary fed with human bacilli tuberculized the guinea pig.

It seems, therefore, that certain birds such as parrots, parrakeets and canaries, very easily infected with the avian virus, possess also a relatively great susceptibility to mammalian bacilli. Hens and other barn-yard fowls are, on the contrary, much less receptive to the latter.

Meanwhile there is no doubt that under certain circumstances human or bovine bacilli may acquire a sufficient virulence even for gallinaceae to cause veritable epidemics among them.

Von Behring,[23] for example, reported that on one farm hens became infected by eating the viscera of a cow which died of generalized tuberculosis. Cultures obtained from the organs of these hens were of the avian type, but their virulence was different; it was very great for the rabbit, for the guinea pig and even for cattle. For the latter they had practically the same virulence as cultures of bovine bacilli from the laboratory of Marburg.

On the other hand, from the experimental side, Nocard,[24] and

[18] Internat. Congr. on Tuberculosis, Paris, 1891.
[19] Centralbl. f. Bakt., 1908, **46**, 461.
[20] Bull. Soc. centr. vétér., 1891, 110.
[21] Ztschr. f. Hyg., 1892, **11**, 445.
[22] Tuberk.-Arb. a. d. k. Gsndhtsamte, 1908, H. 9, 79.
[23] Berl. tierarztl. Wchnschr., 1902, No. 47.
[24] Ann. de l'Inst. Pasteur, 1898, **12**, 561.

later E. Wiener,[25] by cultivating human or bovine bacilli in collodion sacs enclosed in the peritoneum of hens, succeeded in modifying these mammalian bacilli to the extent of rendering them virulent for birds.

Nocard filled his collodion sacs with a thick suspension of a young culture from glycerin potato, left them for several weeks in the peritoneal cavity of his hens, then reinoculated their contents upon potato; put back the culture thus obtained into new sacs which were in turn put into other hens and so on. After three passages the cultures had become sufficiently virulent to seriously infect a rooster.

O. Bang arrived at the same result simply after passing the same virus six times through the body of the hen. He used either pure cultures or the product from grinding the organs of guinea pigs and rabbits previously infected by means of tuberculous tissues. Of 18 strains, 1 was from a horse, 11 from cattle, 2 from the parrot and 4 from human sources. Inoculating intravenously, subcutaneously and intraperitoneally, he succeeded in infecting hens with 12 of these strains, that is to say in 67 per cent of cases. In 6 of these he was able to follow the transformation into avian bacilli.

Zwick and Zeller[26] repeated these experiments utilizing cultures of bacilli from cattle, swine, horses, and human sources, or pieces of organs from rabbits and guinea pigs infected with tissue taken from the preceding animals. They made use of the intravenous, subcutaneous and intraperitoneal routes, as also inhalation and absorption by the digestive tract. They were never able to observe the conversion of mammalian bacilli into the bacilli of fowls. Even in exaggerating all the conditions favorable to this transformation by variously combining the passages, they were no more successful.

Starting from the conception that the lung possesses a peculiar susceptibility to tuberculous infection, in birds as well as in mammals, Bongert attempted to induce pulmonary infection in the pigeon by injecting a pure culture of bovine tubercle bacilli into its larynx, by means of a fine canula. By this procedure he claims to have induced a tuberculosis in his pigeons and to have readily obtained from the lungs of these birds, cultures of tubercle bacilli which are identical with the avian bacillus.

[25] Wien. klin. Wchnschr., 1903, **16**, 581.

[26] Arb. a. d. k. Gsndhtsamte, 1913, **43**, 483; 1914, **47**, 614.

Zwick and Zeller repeated these experiments utilizing the pigeon and the hen. Instead of using the method of Bongert, they exposed the trachea by incision of the overlying skin and entered it with a small needle. They used ground up organs or saline suspensions of tubercle bacilli, the strains coming from cattle, horse, swine and man. They did not succeed in obtaining transformation into the avian type and bacilli collected after passage through the bodies of the birds had preserved all their former virulence for the rabbit and guinea pig.

It must be admitted, in consequence, that the specificity of the avian bacillus for birds, like that of the human or bovine type for mammals, is fairly stable. It is the result of a more or less perfect adaptation which can be realized artificially only with much difficulty. But the success of certain experimenters, where others fail, suggests that there exists in nature a whole series of intermediary types incompletely adapted to one or another species.

E. VIRULENCE OF AVIAN BACILLI FOR MAMMALS

Now and then there have been encountered in various mammals, even in man, cases of spontaneous tuberculosis caused by bacilli presenting all the characters of the avian type. They have been observed the most commonly in the mouse (De Jongh), the rat (L. Rabinowitsch), the rabbit (De Jongh,[27] O. Bang) and swine (Weber and Bofinger, O. Bang, Mohler, Hastings). They have also been noted in the monkey (L. Rabinowitsch), horse (Nocard and Wiener) and bullock (Kruse).

Experimentally, it is very easy to infect mice with avian bacilli, either through inoculation or through ingestion (Römer). The same applies to the rabbit. In this animal, intravenous injection of a small quantity of culture brings about early death with multiplication of bacilli in all the organs, without apparent tubercles. This is the septicemic form known by the name of the type of Yersin, this scientist having been the first investigator to study it well. But with small doses, more or less confluent miliary tubercles develop in the various viscera of the abdominal cavity and in the lungs.

On the contrary, the guinea pig is very resistant to infection with avian bacilli. As a rule there is obtained, by subcutaneous injec-

[27] Ann. de l'Inst. Pasteur, 1911, **24**, 895.

tion, only an abscess at the site of inoculation, with engorgement of the mesenteric and submaxillary glands and with now and then caseous abscesses in the solitary follicles of the intestine. Max Koch however and L. Rabinowitsch, Hastings and Halpin have found strains of avian bacilli whose virulence for the guinea pig was almost as great as that of the human bacillus.

Weber and Bofinger,[28] Titze, De Jongh,[29] Mohler and Washburn,[30] and O. Bang[31] have observed cases of avian tuberculosis in swine, most often localized, but now and again generalized, and they have demonstrated that these animals are readily infected by feeding them with either cultures or cadavers of infected mice or chickens. The English Commission found the avian bacillus in 5 instances among 26 cases of gland tuberculosis in swine.

De Jongh, Steriopolu, Bang, and we ourselves with Guérin,[32] succeeded in infecting young goats by intravenous injection or by ingestion. Contrariwise, we were not able to tuberculize the mammary gland of a lactating goat by introducing cultures directly into the milk ducts by means of a milking tube, although this method of infecting succeeds unfailingly when bovine bacilli are employed.

In horses, intravenous injection of avian cultures is very virulent and a generalized tuberculous process develops with more or less rapidity, according to the dose. Cattle are much less susceptible. Nevertheless, repeated ingestion will produce a mesenteric gland tuberculosis with extensive caseous foci in Peyer's patches. (Kossel, Weber and Heuss,[33] De Jongh, Mettam, Himmelberger).[34]

Avian tuberculosis is therefore transmissible to mammals, but has little tendency to produce in them the acute generalized forms.

It is equally true that it may, under certain exceptional circumstances, be transmitted to man. Lowenstein[35] brought proof of this in two cases of renal tuberculosis in children and in a cutaneous form with abscesses and ulcerations in the nose and intestines. He believes that infection must have resulted from the eating of

[28] Tuberk.-Arb. a. d. k. Gsndhtsamte, 1904, H. 1, 83.
[29] Ann. de l'Inst. Pasteur, 1910, **24**, 895.
[30] U. S. Dept. Agric., Bur. Anim. Indust., Rep., 1910.
[31] Ztschr. f. Infektionsk. . . . d. Haustiere, 1913, **13**, 215.
[32] Ann. de l'Inst. Pasteur, 1905, **19**, 601.
[33] Tuberk.-Arb. a. d. k. Gsndhtsamte, 1904, H. 1; H 3.
[34] Centralbl. f. Bakt., 1914, **73**, 1.
[35] Wien. klin. Wchnschr., 1913, **26**, 785.

infected eggs, either raw or barely cooked. Then too, Weber isolated avian bacilli from the feces of a phthisical patient. Max Koch and L. Rabinowitsch grew them from the spleen pulp of a man dead of miliary tuberculosis and there are still other observations in which bacilli of the avian type have been shown to be infectious for man. (Kruse, 3 cases; Pansini, 1; Lipschutz, 1). In conclusion, Jancso and Elfer[36] found them in pure state in the mesenteric glands of a little girl of 8 years, and B. Lipschutz[37] asserts that avian bacilli are frequently to be encountered in certain forms of cutaneous tuberculosis in man. Inoculation, in such cases, is of no value if the guinea pig is used, whereas it is positive if one makes use of the hen. Herein lies an important fact which invites new research.

F. AVIAN BACILLUS TUBERCULIN

Van Es and Schalk[38] have shown that avian tuberculin can be conveniently used to detect tuberculous infection in hens by intradermic inoculation into the comb or into one of the ears. A local reaction takes place which is quite apparent in 24 hours. It increases during about 48 hours, to disappear after 72 hours. There should be injected 0.05 cc. to 0.07 cc. of 50 per cent avian tuberculin in physiological salt solution. Van Leeuwen[39] finds that at least 80 per cent of chickens giving positive reactions have lesions macroscopically visible. Hens severely infected and cachectic no longer react. He recommends injection into the ear rather than into the comb, in which the connective tissue is more dense.

Tuberculin prepared from avian cultures possesses for tuberculous mammals the same properties as that made from human or bovine bacilli (E. Roux, Maffucci, Babes, S. Arloing). Its toxicity is only slightly less; but animals progressively habituated to one of these tuberculins, resist fatal doses of the avian.

Tuberculous chickens are remarkably resistant. In order to kill them within 2 or 3 hours large doses must be injected (2 to 3 cc. of raw tuberculin); but with smaller amounts focal reactions and general thermic reactions are caused which may persist for several days.

Avian tuberculin may be employed in the same titer as human or bovine tuberculin in testing for tuberculous antibodies in sera.

[36] Beitr. z. klin. d. Tuberk., 1910, **18**, H. 2.

[37] Arch. f. Dermat. u. Syph., 1914, **120**, 387.

[38] Bull. North Dakota Agric. Exper. Sta., 1904, No. 108.

[39] Centralbl. f. Bakt., 1915, **76**, 275.

CHAPTER XXVIII

ACID-FAST BACILLI OF COLD BLOODED ANIMALS

Their Relation to the Tubercle Bacillus

A. THE PISCINE BACILLUS.—ITS CHARACTERS

In 1897, among some carp with large tumors, Dubard, Batai lon and Terre[1] found bacilli resembling that of Koch but growing abundantly upon culture media at temperatures between 10° and 30°C. These fish were living in a hatchery at Velard-sur-Ouche (Cote d'Or), in a little stream into which sputa and dejections from a tuberculous patient had been emptied for several months.

Pieces of the tumors, after 12 to 15 days upon glycerin agar, gave colonies in the form of granular points, dull and of a grayish white color. In broth, at a depth, there was a growth of small flaky granules and a little later a thick film, tending to reach up upon the walls of the flasks.

Preparations from the cultures showed bacilli easily stainable without heat, acid-fast, now homogeneous, now granular, forming bundles or little chains; yet again in filaments.

In the hands of the earliest observers, this organism showed itself pathogenic and capable of tuberculizing all cold-blooded animals upon which it was tried: *carp, triton, frog, toad, turtle, lizard, slow-worm, non-venomous snake* and *viper*.

Cultures made directly from diseased carp were harmless for guinea pigs, rabbits and fowls; but after being passed through several guinea pigs they became virulent enough to produce abscesses with glandular engorgement and a later tuberculization of the liver, spleen and lungs. The authors state that the lesions so obtained were in no way different from those of the most legitimate tuberculosis.

In other experiments, Dubard, Bataillon and Terre think they have succeeded in causing mammalian and fowl tubercle bacilli to take on the property of growing at ordinary temperature upon the

[1] Rev. de la tuberc., 1898, **6**, 13.

usual laboratory media, by passing them through different cold-blooded animals (fish, frogs, lizards). Cultures so modified lose their pathogenic power for animals of constant temperature.

But it should be said here that these facts, which caused considerable stir at the time of their discovery, are today explained very clearly in the light of observations subsequently made by A. Weber and M. Taute,[2] and by N. P. Petrow.[3] These subsequent findings demonstrated the frequent existence of acid-fast bacilli, cultivable at low temperature and living as harmless saprophytes in the body of a large number of species of fish, frogs, lizards, serpents and other cold-blooded animals. The same bacilli are to be found in water, earth, moss, and slime.

It is almost certain that the original carp of Dubard, Bataillon and Terre, which had lived at Velars-sur-Ouche in water abundantly contaminated with dejections and sputa from a phthisical patient, were suffering from parasitic tumors in which was a mixture of true tubercle bacilli of human origin and other acid-fast bacilli pathogenic for fish. For this reason, by inoculating the contents of these mixed tumors, it was possible to obtain mixed cultures capable of infecting warm-blooded animals.

Furthermore, considering that human or avian tubercle bacilli were introduced into the bodies of fish, frogs, lizards, etc., which already contained acid-fasts in abundance, it is not at all surprising that the observers believed that they had recovered from such animals a tubercle bacillus modified as to conditions of culture and virulence. But what they really obtained, on passing their true tubercle bacilli through cold-blooded animals, were cultures of acid-fast saprophytes, with the true tubercle bacilli becoming scarcer and scarcer.

Be that as it may, it has since been demonstrated that the bacillus of Dubard, Bataillon and Terre is not a true tubercle bacillus. It may be pathogenic for certain cold-blooded animals (fish, frogs) but it never becomes so for mammals or for fowls. Nor is it pathogenic for sea fish, or at any rate but very slightly, according to L. Von Betegh.[4]

A. Weber and M. Taute proved that if a culture of human tubercle bacilli is added to the water of an aquarium containing frogs, one

[2] Tuberk.-Arb. a. d. k. Gsndhtsamte, 1905, II. 3, 110.
[3] Centralbl. f. Bakt., 1907, **43**, 349.
[4] Ibid., 1910, **53**, 374.

can after 14 days,—the frogs being washed and immersed for 13 seconds in boiling water before autopsy,—recover from their visceral organs bacilli which are virulent for guinea pigs, whereas they cannot be recovered from the organs of control frogs left but 10 minutes in the contaminated aquarium.

According to them, one is justified in distinguishing the bacillus of tuberculosis of cold-blooded animals from the ordinary saprophytes of moss, earth, slime, etc. The latter are not pathogenic for the frog, whereas the type of Dubard-Bataillon-Terre causes death of the frog 2 to 4 weeks after the inoculation of a few centigrams of culture into the dorsal lymphatic sac. In the frog's liver these various types of acid-fasts are often found associated, some pathogenic, others innocuous.

B. ACID-FAST BACILLI OF REPTILES AND BATRACHIA

Along with the bacillus of Dubard-Batillon-Terre there should be placed the bacillus which W. K. Sibley[5] described in 1889, found in a common snake (*Tropidonotus natrix murorum*) in some small tumors the size of hazel-nuts, some adherent to the skin, others disseminated in the liver, kidneys and intestine; the organism which Hansemann[6] discovered in the abdomen of a python, in the neighborhood of the pancreas; those of E. Kuster,[7] isolated from the livers of three frogs; and that which Friedmann[8] found, at the Berlin Aquarium, in the right lung of a turtle which was fed by a tuberculous employee.

The bacillus of Friedmann, about which there has been much question because of the attempts, however unsuccessful, on the part of this author to utilize it for antituberculous vaccination (*see Chapter XLII, C, 8*), grows equally well at 22° and 37°C. When inoculated into the turtle, lizard, or common snake, it produces a miliary tuberculosis. The slow-worm (*Anguis fragilis*) is very susceptible to it and dies in from 7 to 54 days with a generalized infection. This same bacillus multiplies also very actively in the frog.

Warm-blooded animals are refractory or present only a caseous

[5] Virchow's Arch., 1889, **116**, 104.
[6] Centralbl. f. Bakt., 1903, **34**, 212.
[7] München. med. Wchnschr., 1905, **52**, 57.
[8] Deutsch. med. Wchnschr., 1903, **29**, 464; 1904, **30**, 166.

focus at the point of inoculation. The guinea pig, injected with very large doses intraperitoneally, succumbs in 4 to 8 days and a beginning formation of tubercles is then found in the peritoneum, along with caseous masses. If the animal survives, he may tuberculize himself with the formation of typical giant cells, and the lesions end by healing without leaving any traces.

C. ATTEMPTS AT TRANSFORMATION OF TUBERCLE BACILLI OF WARM-BLOODED ANIMALS INTO BACILLI OF THE PISCINE TYPE

Infection of cold-blooded animals by human, bovine or avian tuberculous virus has been the object of a great many researches. Morey[9] states in his thesis that Verga and Biffi attempted in vain to accomplish it in frogs as early as 1868. Despeignes[10] in 1891 made similar attempts in frogs, salamanders and fish. Combemale[11] inoculated and fed carp for several months with tuberculous sputa and arrived at the conclusion that the bacillus remains morphologically unchanged in the body of these fish but that it gradually loses its vitality and virulence.

Hormann and Morgenroth[12] carried out the same experiment with gold-fish and in their bodies recovered living bacilli inoculable into guinea pigs after 7 months.

Lortet and Despeignes,[13] going back to the experiments of Pasteur on the spores of anthrax, found likewise that earth-worms, collected in soil where tuberculous cadavers had been buried, preserve living and virulent bacilli in their digestive tracts for a very long time and disseminate them with their dejections.

Ramond and Ravaud,[14] Lubarsch and Mayer, Auché and Hobbs,[15] Ledoux-Lebard,[16] Moeller, A. Weber and Taute, Gozo Moziya,[17] L. Von Betegh,[18] Dieudonné and other workers, endlessly varying the conditions of their experiments, have never been able to furnish

[9] Thèse, Lyon, 1900.
[10] *Étude sur la tuberculose.* 1891.
[11] Congrès des Soc. Savantes, 1893.
[12] Hyg. Rundschau, 1899, **9**, 857.
[13] Compt. rend. Acad. des sci., 1892, **114**, 186.
[14] Compt. rend. Soc. de biol., 1898, **50**, 589.
[15] Ibid., 1899, **51**, 816; 817; 825.
[16] Ann. de l'Inst. Pasteur, 1900, **14**, 535.
[17] Centralbl. f. Bakt., 1907, **45**, 294; 1909, **51**, 480.
[18] Ibid., 1910, **54**, 211.

proof of a true adaptation of the virus of warm-blooded animals to the economy of cold-blooded vertebrates.

Sorgo and Suess[19] believe that they succeeded. They injected human bacilli into two slow-worms and twenty serpents of various species (*Tropidonotus natrix*, *Coluber esculapii*, *Zamenis viridiflavus*). In the two slow-worms and in four of the snakes, they were able to find a few lesions of caseous degeneration at the point of inoculation and, in one case, the cultures obtained had taken on some characters identical with those of the bacilli of cold-blooded animals. They had become pathogenic for serpents and harmless for guinea pigs.

E. Herzog[20] claims also to have obtained positive results. He introduced mammalian bacilli into the dorsal sac of frogs, and kept the latter at laboratory temperature, giving them no other nourishment than what they could extract from the daily renewed water. After varying intervals, he killed the frogs, washed and ground up the livers and injected them into the peritoneal cavity of guinea pigs. Other organs were reserved for microscopic examination. These experiments showed that the human bacillus preserves its virulence in the body of cold-blooded animals for more than 120 days. The guinea pigs however die the more slowly the longer the bacilli have remained in the body of the frogs.

Two interpretations come to mind: either the bacilli diminish in number in the body of the frog, so that they become less and less harmful; or else, their number remaining unchanged, they undergo an attenuation of their original virulence. From calculations which he made on the number of bacilli which might be contained in the liver, as well as from microscopic examination, Herzog concludes that the first hypothesis must be rejected and, if the guinea pigs die after a longer and longer delay, it is because the bacilli are becoming less and less virulent. During the processes of attenuation in their passage through the frog, the bacilli are still capable of producing in time a generalized tuberculosis in the guinea pig; but, if the passages are multiplied, a race of bacilli innocuous for the guinea pig is ultimately obtained.

A. Weber and M. Taute[21] do not accept this interpretation of Herzog. They think, on the basis of their own experiments, that the bacilli found in the livers of frogs, often in very large number, and

[19] Centralbl. f. Bakt., 1907, **43**, 422; 529.
[20] Ibid., 1903, **34**, 535; 675.
[21] Deutsch. med. Wchnschr., 1904, **30**, 1019.

which are not virulent for the guinea pig, are simply acid-fasts of the piscine group, which have nothing in common with tuberculous virus.

Bertarelli and Bochia,[22] refusing completely to subscribe to the idea of transformation, have found that human, bovine, and avian bacilli inoculated into carp or lizards (*Varanus varius*), actually multiply in these animals so that at the end of 8 months they are present in numbers incomparably greater than when injected, and their original virulence is unaltered. Therefore, *no attenuation of bacilli of warm-blooded animals is produced by passages through cold-blooded vertebrates.*

D. NON-IDENTITY OF TUBERCULOUS VIRUS OF WARM-BLOODED ANIMALS AND ACID-FAST BACILLI OF COLD-BLOODED ANIMALS

From all the foregoing facts the conclusion stands out that *the tuberculous virus of mammals and that of birds are very different from the piscine bacillus,* which is itself but slightly pathogenic for fish. It has been in no wise demonstrated that the latter can be converted into a bacillus virulent for warm-blooded animals, nor is it proved that tubercle bacilli virulent for warm-blooded animals can be modified or transformed by their more or less prolonged sojourn in the body of the cold-blooded animals.

In addition, *the specificity of the piscine bacillus is affirmed by the fact that it is incapable of serving as antigen for the fixation of true tuberculous antibodies, as in the reaction of Bordet-Gengou.*

Furthermore glycerinated extracts obtained by evaporating these cultures, and the bodies of the bacilli themselves, isolated from the cultures, are devoid of any toxicity for tuberculous guinea pigs.

Terre, Ramond and Ravaud, Ledoux-Lebard, and Krompecher, believed, it is true, that they had prepared an active tuberculin from the original carp bacilli of Dubard, Bataillon and Terre. But Pinoy and Burnet in Borrel's laboratory at the Pasteur Institute, as well as A. Weber and M. Taute,[23] were never successful. Borrel[24] himself found that the bacilli brought to him by Dubard, were, after desiccation, harmless for the tuberculous guinea pig when inoculated subcutaneously in a dose of 600 mgms., whereas 20 mgms. of bacilli of the human type were sufficient to kill a tuberculous control under the same conditions.

[22] Tuberculosi, 1909/10, **2**, 305.

[23] Tuberk.-Arb. a. d. k. Gsndhtsamte, 1905, H 3, 110.

[24] Bull. de l'Inst. Pasteur, 1904, **2**, 409; 457; 505.

CHAPTER XXIX

THE ACID-FAST PSEUDO OR PARATUBERCLE BACILLI

Soon after the discovery of the bacillus of Koch, Zahn[1] drew attention to the presence, in the sputum of a non-phthisical subject, of bacterial elements having about the same morphological appearance as the tubercle bacillus and which, like the latter, were resistant to decolorization by acids. Alvarez and Tavel,[2] Matterstock, and Bitter,[3] found other and similar bacteria in vulvar or preputial smegma; Gottstein in cerumen; Cramer and de Giacomi,[4] in the normal contents of the human intestine. Fraenkel,[5] Pappenheim, and L. Rabinowitsch[6] remarked their frequency in the expectoration from cases of pulmonary gangrene; Laabs, and Moeller[7] in saliva and in the cutaneous secretions of healthy subjects; Dietrich in the fluid from a suppurative cyst of the ovary; W. Ophuls[8] in the pus of an abscess in the iliac region; Petri, Rabinowitsch, Hormann and Morgenroth, Grassberger, Herbert, Wessenfeld, Korn, Ascher, Coggi, Herr and Beninde, Mlle. Tobler,[9] and Jean Binot[10] in milk, cream and butter; Moeller in dung and fodder, Houston in the sewage of London; Séverin, Cappaldi, and Ferran in dejections from horses, cattle and man.

Suffice it to say that these acid-fast bacteria are extremely common in nature. They are encountered under all sorts of circumstances, in soil, in water, in dusts, on the skin and on the mucous membranes of man and healthy animals. Only a few are virulent, and among

[1] Thèse, Tübingen, 1884.
[2] Arch. de physiol. normale et path., 1885, p. 303.
[3] Virchow's Arch., 1886, **106**, 209.
[4] Fortschr. d. Med., 1883, p. 145.
[5] Berl. klin. Wchnschr., 1898, **35**, 246; 880.
[6] Deutsch. med. Wchnschr., 1900, **26**, 257.
[7] Ztschr. f. Hyg., 1889, **32**, 205.
[8] J. Med. Research, 1904, **11**, 439.
[9] Ztschr. f. Hyg., 1901, **36**, 120.
[10] Arch. de parasitol., 1903, **7**, 306.

the latter the best known are the *tubercle bacillus of Koch*, the bacillus of *leprosy* discovered by Hansen, the bacillus of *bovine enteritis* of Johne, and that of *rat leprosy* of Stéphanski.

Of the others, the very great majority are essentially saprophytes and probably play an important rôle in the decomposition of fatty substances. Certain of them however are perhaps capable also of becoming pathogenic. At any rate they are encountered in lesions in the formation of which they appear to be playing some rôle in certain abscesses for example, and when inoculated into animals in pure cultures, there are some which produce local disorders consisting of exudative inflammations with or without false membranes or nodules. The local lesions are caseous at times, are lacking ordinarily in giant cells and have no tendency to become generalized.

Most of these organisms are easily cultivated. Almost all of them grow rapidly—sometimes in a few hours—on the common media and at temperatures varying from 15 to 38°C.

On peptone agar, with or without glycerin, upon potato, on gelatin broth and upon carrots and beets the cultures grow exuberantly in thick layers in dry or fatty folds. On fluid media, they form both a surface film which is more or less wrinkled and thick, and a sediment. They do not coagulate milk, nor do they liquefy gelatin. Some give forth an ammoniacal odor; others form a little indol.

Almost all have a color varying from yellow to brick red. Pigment production is favored, according to Thévenot,[11] by glucose, and mannite; by glycerin according to P. Courmont.

These bacteria are all non-motile; they are Gram-positive; to a greater or lesser degree they resist decolorization by acids or by immersion in boiling water (2 to 2½ minutes, G. Gair) and in form they are at times identical with true tubercle bacilli, now thicker or shorter, now long and branched, at times radiating or in masses like *Actinomyces*.

They owe their property of acid-fastness to the fatty acids and wax which enter into the composition of their protoplasm as is the case with the bacillus of Koch.

[11] Compt. rend. Soc. de biol., 1906, **61**, 223.

A. SPECIAL CHARACTERS OF THE PRINCIPAL VARIETIES OF PARATUBERCLE BACILLI

1. *Acid-fast bacilli encountered normally in man*

The most common are those of *smegma*, which, says, Moeller can be cultivated upon human serum; next those found by Rabinowitsch in pulmonary gangrene; next those found by Laabs, and by Karlinski upon the nasal mucosa, in comedones and in the sweat

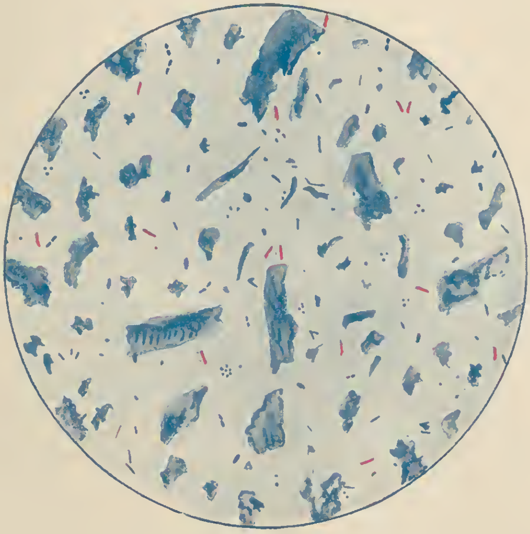


FIG. 25. ACID-FAST BACILLI IN FECAL MATTER OF CATTLE
Imm. $\frac{1}{18}$, oc. comp. 6, Reichert

glands of the feet; then those found by Bienstock and Gottstein in ear cerumen.

These bacilli are not pathogenic for either rabbits or mice, but in guinea pigs, when inoculated intraperitoneally, they may produce exudative inflammatory lesions with the formation of false membranes or granular nodules in the region of the spleen and liver, and engorgement of the glands. No giant cells are to be found.

2. Acid-fast bacilli of soil, sewage and excrement (fig. 25)

The timothy or grass bacillus of Moeller,[12] of which there are two varieties, and the mist bacillus or the bacillus of dung, isolated by the same investigator, are the most interesting of this category.

The grass bacillus is obtained by inoculating glycerin agar plates with field grasses (chiefly the stem and the head of the grass) macerated at 37°C. in sterile water from 8 to 14 days. Growths upon glycerin potato or upon agar are wrinkled, scaly, puffy and of a grayish white or reddish color. If inoculated into the peritoneal cavity of the guinea pig, the animal dies at times in one or two days and bacilli are then found in the blood. At other times the guinea pig emaciates and dies in from 5 to 6 weeks showing at autopsy some whitish nodules in the peritoneum, false membranes adherent to the intestinal loops, to the omentum and to the mesentery, enlarged and red suprarenal capsules and now and then nodules and cavities in the lungs. Round about these cavities are seen formations which resemble giant cells and which are surrounded by fibrous tissue. The miliary tubercles are composed of epithelioid cells with vesicular nuclei surrounded by a zone of lymphocytes. Between and within these tubercles are to be found the grass bacilli more or less branched and with terminal swellings sometimes in the form of a star.

If injected into the veins of a rabbit, this bacillus often produces lesions singularly like tuberculosis: giant cells, epithelioid cells and caseation almost always typical.

In one of our experiments C. Guérin[13] and I introduced, by means of a milking tube, a dose of 0.70 gm. of a fresh culture of the grass bacillus into each teat of a goat about to have young. Four days later two goats were born. The latter from birth were drinking milk which was very rich in acid-fast bacteria. At the end of two weeks, an examination of the milk showed that the bacteria had disappeared.

Guinea pigs inoculated with 0.5 cc. of milk of the early days died cachectic two months later, with small abscesses at the point of inoculation but without visceral lesions other than an adhesive peritonitis.

The two new-born goats grew normally. One was killed on the

[12] Centralbl. f. Bakt., 1901, **30**, 513:—Deutsch. med. Wchnschr., 1898, **24**, 376; 1902, **28**, 466; 718.

[13] Ann. de l'Inst. Pasteur, 1905, **19**, 605.

forty-fifth day. At autopsy the mesenteric gland chain was found much enlarged. The glands themselves, although large and soft, showed no tubercle formation on section nor any thickening of the cortical layer. They were full of leucocytes among which there were no stainable bacilli. The other organs and the intrathoracic lymphatic system were perfectly normal.

Rodet and Galavielle[14] fed a calf daily during several weeks with a large quantity of grass bacilli admixed with its food. The animal on being killed presented an engorgement of the mesenteric glands. It had been tested twice with tuberculin and found negative.

Cultures of the grass bacillus are therefore harmless for the calf, and animals inoculated remain *non-sensitive to tuberculin prepared from the bacillus of Koch.*

The *mist bacillus,* discovered by Moeller in cow dung, is totally resistant, particularly when in young cultures, to decolorization by acids and by alcohol. It grows like the timothy bacillus upon agar or glycerin potato, producing in 4 to 5 days at 37°C. an ochre pigment. Its effects upon the guinea pig or rabbit are approximately the same as those of the grass bacillus, but less marked.

3. Acid-fast bacilli of milk and of butter

There exists a fairly large number of varieties which will be found well described in the thesis of M. Potet.[15] The most important go by the following names:

The Bacillus of Petri;[16]
B. of Rabinowitsch;
B. type I of Korn;[17]
B. type II of Korn;
B. of Coggi;
B. of Tobler[18] (types I to V);
B. of Markl;
B. of J. Binot;
B. of milk, of Moeller.

[14] Compt. rend. Soc. de biol., 1905, **59**, 552.
[15] Thèse, Lyon, 1902.
[16] Arb. a. d. k. Gsndhtsamte, 1898, **14**, 1.
[17] Arch. f. Hyg., 1899, **36**, 57:—Centralbl. f. Bakt., 1899, **25**, 532.
[18] Ztschr. f. Hyg., 1900, **36**, 120.

All these bacilli grow readily upon the usual media, whether glycerinated or not, at room temperature or in the incubator. Those of Mlle. Tobler liberate a little ammonia. None of them liquefy gelatin nor coagulate milk.

When inoculated into animals, especially in a mixture with butter and by way of the peritoneum, they are capable of producing according to the dose, either generalized infections or a peritonitis with false membranes and a nodular exudate.

M. Beck[19] described under the name of *B. tuberculoid I* and *II* two types of acid-fasts which he isolated, the first in 1897 by inoculating butter into guinea pigs, the second in 1901, from the tonsils of a woman who died of pulmonary tuberculosis.

Cultures of the first, especially when grown on broth, gave a characteristic odor of trimethylamin. They grew quickly at 37°C. and even at 18°C. upon ordinary agar.

Those of the second grew well on glycerinated media between 25 and 40°C. Broth cultures were cloudy. Sera of phthisical patients agglutinated them perfectly in a 1 to 20 dilution.

Tuberculoid I is almost devoid of pathogenicity except when injected with grease or butter. Swellings over the peritoneum and the surface of the viscera then result; they contain bacilli arranged in lines between the white cells.

Tuberculoid II is much more virulent. On subcutaneous inoculation it usually kills the guinea pig in 8 to 10 weeks, with swelling of the spleen, necrotic foci in the edges of the liver, and miliary tubercles in the lungs. Intraperitoneal injection of 0.1 gm. of culture leads to a generalized tuberculosis. By ingestion, in the guinea pig, tumefaction and caseation of the mesenteric glands are produced. By inhalation, this bacillus is innocuous.

Organs of infected guinea pigs or rabbits can never be inoculated serially. Corresponding glands may caseate, or even suppurate, but no bacilli are found in the organs.

No giant cells are to be seen in the sections; only a coagulation necrosis with masses of leucocytes about the foci. Bacilli are fairly rare, almost always accumulated between the cells.

Mice and birds are refractory; calves are equally so and are in no wise vaccinated against the true tubercle bacillus.

[19] Tuberk-Arb. a. d. k. Gsndhtsamte, 1905, H. 3, 145.

B. DIFFERENTIAL DIAGNOSIS OF PARATUBERCULOUS ACID-FASTS FROM TRUE TUBERCLE BACILLI

Moeller[20] writes: "In any case where, in the absence of all physical signs, the diagnosis of pulmonary tuberculosis rests solely on the presence of acid-fast bacilli in the sputum of the patient, one must employ the following procedure based upon the slowness of growth of the tubercle bacillus and its special requirements as regards temperature, if one is to be certain of the diagnosis:

"The sputum in question is added to ordinary broth and the mixture kept at 30°C. If the acid-fast bacilli multiply under these conditions one can be certain that they are not true tubercle bacilli. Now and again however, with true tubercle bacilli, one may observe a multiplication of bacilli in the sputum mixed with certain culture media and maintained at incubator temperature. This multiplication may be explained by the presence in the sputum of certain substances coming from the human body and bearing some resemblance to globulin; but the multiplication in these cases is but slight and ceases after 48 hours, whereas if the pseudobacilli of tuberculosis are present the increase takes place at 30°C."

Furthermore one may make use of the method of Spengler, as proposed by S. Piatkowski,[21] taking advantage of the relatively slight susceptibility of acid-fasts to formaldehyde. The procedure is then as follows:

The bacterial mixture to be examined is emulsified in 10 cubic centimeters of water or sterile broth; two or three drops of formol are added and the contents of the tube shaken briskly. Half an hour later the fluid is planted upon plain or glycerin agar, and this operation repeated every quarter of an hour. As a result, there is obtained a certain number of tubes which furnish a pure culture of acid-fast bacilli, all other bacteria having been killed by the formol.

It is often useful to have recourse to these procedures, controlled by guinea pig inoculation, in order to avoid errors of diagnosis in certain doubtful cases. R. Milchner[22] has given a curious example of this sort in a patient of 52 years in whom physical examination, constitutional symptoms and repeated hemoptyses denoted tuberculosis,

[20] Centralbl. f. Bakt., 1901, **30**, 513.
[21] Deutsch. med. Wchnschr., 1904, **30**, 878.
[22] Ibid., 1903, **29**, Ver.-Beil., 130.

although the general condition continued apparently excellent. The sputum contained an abundance of acid-fast bacilli, but gave only negative results on animal inoculation. Following a new and final hemoptysis the sputum, still rich in bacilli, became fetid, which aroused suspicion of a dilatation of the bronchi, and, as a matter of fact at autopsy there was found an enormous bronchiectasis. Macroscopic examination of the lungs, as well as of the peribronchial lymph nodes, although made with the greatest care, did not disclose the slightest trace of caseation, nor even of induration.

In the sections, Milchner could not find a single bacillus because of an error which should be avoided; the tissue had been fixed either in alcohol or formol, and when tissue containing acid-fasts is submitted to the action of one or the other of these reagents the bacilli lose the property of taking the stain.

F. W. Twort and G. L. Y. Ingram[23] recommend, as a differential culture medium, the egg medium of Dorset into which are mixed the bodies of acid-fast bacilli killed and, preferably, dried. The human or bovine tubercle bacillus does not grow under these conditions, while the grass bacillus, for example, furnishes very abundant cultures. It is not even necessary to use whole bacteria: glycerinated saline extracts and alcoholic extracts are said to serve equally well.

Aside from cultural characteristics, it is possible to differentiate paratubercle bacilli from true tubercle bacilli;

1. By the study of their secretory products;
2. By agglutination reactions with serum of vaccinated animals;
3. By their pathogenic properties.

The secretory products of paratubercle acid-fasts are practically devoid of toxicity for tuberculous animals.

Borrel, Pinoy and Burnet[24] at the Pasteur Institute were never successful in obtaining tuberculin with glycerin extracts of cultures of the *grass* bacillus of Moeller, nor with that of butter isolated by J. Binot. Nor did they succeed in killing tuberculous guinea pigs by injecting them with large doses of the bacterial bodies of the same bacilli.

Agglutination reactions, studied by Defalle,[25] Paul Courmont

[23] Proc. Roy. Soc., Lond., 1912, s. B., **84**, 517.
[24] Bull. de l'Inst. Pasteur, 1904, **6**, 420.
[25] Ann. de l'Inst. Pasteur., 1902, **16**, 595.

and Potet,[26] with the bacillus of Binot and Korn I, furnish no definite means of identification. The acid-fast bacilli, in their experience, are but slightly agglutinable with homologous sera and not agglutinable at all with the sera of tuberculous subjects. Even the sera of tuberculous animals prepared with Arloing's homogeneous bacillus and possessing for it a very high agglutinating titre, have practically no effect upon the acidophiles.

As for pathogenic properties, it may be said, in a general way, that the paratubercle acid-fast bacilli are, in small doses, most often innocuous for experimental animals. Some of them, as we have said, are capable of inducing the formation of tubercles just as do many more or less irritant foreign bodies like lycopodium powder or the larvae of certain parasites. But these tubercles do not tend to produce extensive lesions. *They can never be reinoculated in series into other animals,* and, when injected intravenously, into the rabbit for example, the acid-fast bacilli show a peculiar aptitude, like the mycoses, for localizing in the kidneys.

C. ACTION OF PARATUBERCLE BACILLI UPON THE EVOLUTION OF TUBERCULOSIS

Many investigators have endeavored to vaccinate animals against tuberculosis by injecting them preliminarily with cultures of paratubercle bacilli. F. Klemperer[27] made numerous attempts of this sort. He thought that he succeeded in obtaining an appreciable resistance to virulent test inoculations in guinea pigs by injecting them 21 times intraperitoneally, during a period of three months and a half, with one loop of culture of the milk bacillus. However it was only a matter of death being retarded as compared with the controls.

Larger animals, like the goat and the calf, are in no sense vaccinated by intravenous injection of the grass bacillus, nor do they, by virtue of these injections, acquire even the slightest resistance to virulent inoculations of bovine tubercle bacilli.

I was able to show, with C. Guérin,[28] that the injection of this same grass bacillus is harmless for young goats; but it is capable of producing a mesenteric adenopathy which, though intense, has no vaccinating power against tuberculosis. Our experiments were as follows:

[26] Arch. de méd. expér., 1903, **15**, 83.
[27] Ztschr. f. klin. Med., 1903, **48**, 250.
[28] Ann. de l'Inst. Pasteur, 1905, **19**, 601.

Two goats, born April 27, 1905, were given 0.05 gm. of a fresh culture of the grass bacillus through an esophageal tube on each of the following days: April 28, May 5, 6, 15 and 17. The animals remained in perfect health. One of them was killed on July 12. The mesenteric gland chain was found considerably enlarged, the nodes forming a large strand. Certain of them were as large as hazel-nuts. On section small white islands of sclerosis were to be seen in the cortex. There were no stainable bacilli. Inoculation of one of these glands, after grinding, into the peritoneum of 4 guinea pigs produced no disease in the animals. The liver, spleen, lungs, and thoracic glands showed no lesions.

The other goat, which was given 0.05 gm. of culture of the grass bacillus on May 5, 6, 15 and 17, received through an esophageal tube on June 13, 14, 15 and 16 an additional 0.05 gm. of a fresh bovine culture. On July 9 following he began to lose weight and to cough, and was killed on July 10. The mesenteric glands were only a little swollen, but showed a fairly large number of small caseous tubercles in the cortex. The other abdominal organs were unaffected. The lungs, to offset this fact, appeared sown with tubercles and almost entirely hepatized. The glands of the posterior mediastinum were enormous; the peribronchial and retropharyngeal glands were still uninvolved. A piece of ground-up lung tissue was inoculated subcutaneously into two guinea pigs which died after 45 days with generalized tuberculosis.

Such results seem to dispose of the idea, as expressed by certain authors, of a common origin of paratubercle and true tubercle bacilli.

The para or pseudo-tuberculous acid-fasts belong perhaps to the same botanical family as the *Bacillus tuberculosis*, that of the *Oospora;* but they constitute thoroughly distinct species, with nothing to warrant admission of the possibility of a reciprocal transformation.

D. EXPERIMENTS OF J. FERRAN ON THE TRANSMUTATION OF THE TUBERCLE BACILLUS INTO A SAPROPHYTE

J. Ferran[29] (of Barcelona), from a series of experiments (the beginning of which will be found in a note by him before the Academy of Sciences of Paris on August 6, 1897), believes that he has observed the transformation of the tubercle bacillus into a true motile and

[29] Rev. de méd., 1901, **21**, 1009; 1902 **22**, 54:—Arch. gén. de méd., 1903, i, 3.

ciliated saprophyte, having completely lost its acid-fast property and assumed the aspect and staining reactions of *Bacillus coli.*

By planting the tubercle bacillus upon broths with a lower and lower content of glucose, glycerin and peptone, and by shaking the cultures daily (as did S. Arloing later), Ferran obtained strains which were emulsive and homogeneous, with individual bacilli both ciliated and motile. These bacteria then grow at ordinary temperature, acidify lactose media, give an indol reaction on peptone media, and are said to be agglutinated by the sera of tuberculous subjects. When grown upon fluid serum of horse, sheep or bullock at laboratory temperature, and afterward injected subcutaneously in fractional and repeated doses into the guinea pig, they cause an initial edema and then a phlegmon. Bacilli isolated from the edema exudate or from the pus and transplanted upon liquid serum, produce a growth which has the very characteristic odor of human sperm and gives reactions which Poehl attributes to *spermine.* "It appears," says Ferran, "that it is the leucocytes in the inoculated material which stimulate the 'spermogenic' function of the latter."

According to Ferran, it is possible to restore to this colon bacillus variety of the tubercle bacillus the latter's tuberculogenic action. To that end it is necessary to enhance its virulence by means of serial inoculations from guinea pig to guinea pig, and to continue them until a first animal dies, then a second, etc. The inflammatory phlegmonous lesions are succeeded by tubercle-formed lesions and, when tubercles appear, the tubercle bacillus is recovered therein with its normal acid-fast characters.

In 1903, J. Auclair[30] published a memoir in which he confirmed in part the researches of J. Ferran. Starting with an authentic culture of the bacillus of Koch, he too obtained a bacterium which developed in homogeneous culture, grew rapidly upon ordinary media, especially at 37°C., and resembled *Bacillus coli.* This variety of saprophyte was said to be no longer virulent for the guinea pig and rabbit: the bacilli appeared to be rapidly destroyed in the tissues. However after repeated inoculations the animals finally died after several months with cachexia but without nodular lesions in the organs.

Auclair was not successful in inversely transforming his saprophytic bacillus into the tubercle bacillus. He thinks this is perhaps due to

[30] Arch. de méd. expér., 1903, **15**, 469.

the fact that he pushed the transmutation further than did J. Ferran, so that the saprophytic characters became more firmly fixed.

This rather bold transmutation hypothesis has not since been verified, despite efforts on the part of many workers. Meanwhile J. Ferran is as convinced as ever of the correctness of his observations, and we shall see in another chapter (XLII) that he is persevering in his attempts to utilize his non-tubercle-forming bacteria derived from the tubercle bacillus[31] as a *vaccine* against tuberculosis.

[31] The study of the zoögleic pseudo-tuberculosis of Malassez and Vignal, as well as that of the various pseudo-tuberculoses produced by non-acid-fast bacteria (pseudo-tuberculosis of rodents or pseudo-tuberculosis of man) is not within the scope of this work. The reader desiring to know the works on these pathogenic agents will find a good bibliography in the articles of K. Saisawa, Ztschr. f. Hyg., 1913, **73**, 353; 401.

PART THREE

Processes of Defense and the Diagnosis of Tuberculous Infection

CHAPTER XXX

REACTIONS OF DEFENSE ON THE PART OF THE BODY AGAINST TUBERCULOUS INFECTION

Cellular Enzymes

When a tubercle bacillus gains entrance into a susceptible host which is free from previous tuberculous infection, no matter whether this penetration occurs by way of the lymphatics—as is most often the case in natural infection—or by way of the blood stream, for example following an intravenous inoculation, it immediately becomes the prey of a phagocytic polynuclear leucocyte. But the leucocyte lacks the power to destroy the bacillus, because the latter is so remarkably well protected against the digestive action of the cellular juices by its content in chitin, waxes and fats.

If this bacillus is lacking in vitality or virulence (bacilli dried, modified by light rays, by heat or by chemical substances, bacilli from other species of animals), it may be borne along for a shorter or longer period in the circulatory system or may remain fixed in some lymphatic gland near the point of inoculation. But ultimately it is expelled from the body, either in the pus of a cold abscess or with the biliary pigments by way of the liver and intestines.

If this bacillus be living and virulent, it multiplies in the phagocyte which serves as host, there secretes its poisons, and kills the cell. The destruction of the leucocyte sets free in the body fluids, in addition to the mass of young bacilli developed in its protoplasm, débris of degenerated cell nuclei and complex *enzymes* of leucocytic origin.

Among these enzymes, there are some which remain as they were within the normal leucocyte (*protease, lipase, amylase, oxydase, complement,* etc.). Others are formed before the death of the cell and are abnormal products resulting from the life of the bacillus in the protoplasm of the parasitized cell. The rôle of the latter enzymes in the further evolution of the disease is of altogether prime importance. It seems indeed that they are the essential factors in the *sensitization* of the infected host and also in the *resistance* which

that organism, as we shall see later, is capable of acquiring against reinfections. As yet, indeed, we understand them but very imperfectly, although certain factors stand revealed by effects whose intensity can be measured. Such is the case at least with the *coagulins* and *lysins*.

A. CELLULAR PROTEASE

The researches of Fr. Muller, E. Fischer, of E. Abderhalden and those of Eug. Opie and Bertha Barker,[1] have shown that the polynuclear leucocytes of man and other mammals, particularly the neutrophiles (Muller and Jochmann), contain an enzyme which digests albuminoid substances as does pancreatic trypsin in a weakly alkaline medium. The lymphocytes derived from inflammatory exudates likewise contain another enzyme said to be active in a weakly acid medium (1/100 normal HCl). It is known that the same holds true for the lymphatic glands and for the pulp of certain organs such as the bone marrow, liver, spleen, kidneys, and also the globulins of normal serum. To these enzymes are due the phenomena of autolysis (Hedin).[2] But, along with the *leucoprotease*, there exists in normal serum an *antiprotease* which is destroyed by heat only at a temperature of 75°C. and which is generally found in such excess in the living body as to offset the digestive action of protease upon the cells. According to Opie and Barker the serum of the rabbit is especially rich in antiprotease and poor in protease and because of this peculiarity they explain the fact that in this animal there is never observed a suppuration going on to liquefaction of the tissues.

The same investigators have studied the properties of these enzymes and antienzymes in tuberculous organs and exudates. From what they have observed, the protease of epithelioid cells is weakly digestive for albumin in an acid medium and more weakly still in a neutral medium. This protease is said to be different from that of the polynuclear leucocytes, and its action is not prevented by the serum of a tuberculous exudate called forth by injecting bacilli into the pleural cavity of a dog, whereas, on the contrary, it is inhibited by serum from the blood of the same animal. Two observations would indicate that this proteolytic activity disappears immediately before death and that it is manifested only in exudates

[1] J. Exper. Med., 1907, **9**, 207; 1908, **10**, 645; 1909, **11**, 686.

[2] J. Physiol., 1904, **30**, 155.

of animals inoculated with a bacillus to which they are relatively resistant, while it is totally lacking in animals inoculated with a very virulent bacillus (bovine bacillus for the dog).

Human pleuritic exudates, in the experience of Opie and Barker, were found to be inactive.

It does not seem therefore that either the *leuco* or *lympho-protease* enters in any appreciable manner into the digestion of the tubercle bacillus by the leucocytic protoplasm, so marvelously is the bacillus protected against these digestive actions by its waxy fatty ectoplasm. Only the enzymes acting upon the latter, among which *lipase* has been best studied, can play a rôle of any importance.

B. THE CELLULAR LIPOLYTIC FERMENTS

The leucocytes, blood serum and cells of certain organs, above all the glandular organs, and particularly the pancreas, the spleen and lymphatic glands, normally contain enzymes which have the property of breaking down fatty substances into fatty acids and glycerin. In the opinion of Bergel, these enzymes are especially abundant in the *lymphocytes*.

These enzymes are called *lipases*. Hanriot demonstrated them to be reversible: in alkaline medium they decompose fats into fatty acids and glycerin but in acid medium on the contrary they combine fatty acids and glycerin to form neutral fats.

N. Fiessinger and Marie,[3] using the methods proposed by Hanriot and Camus[4] and by Clerc, sought to determine the lipolytic power of lymphatic glands, spleen and bone marrow for monobutyrin and neutral fat of butter. They found that the juices of these organs possess a lipolytic activity which is greater in the sheep and in the calf than in the guinea pig and rabbit. The lipase is said to be particularly abundant in lymphoid tissues, spleen and glands. It is also found in lymphocytic exudates (pleural and ascitic fluids, hydrothorax, hydrarthrosis), while it is lacking in the polynuclear exudates of acute suppurations.

According to Fiessinger and Marie,[5] the lipase extracted from tuberculous suppurations exerts a characteristic action upon the tubercle

[3] Compt. rend. Soc. de biol., 1909, **67**, 107; 177.

[4] Compt. rend. Acad. des sci., 1897, **123**, 831.

[5] Rev. de la tuberc., 1910, **7**, 186.

bacillus. By treating suspensions of the latter with definite quantities of lipase-containing fluid for 24 hours at 52°C. and then centrifugating, the fatty content of the bacillus seems to be attacked, since the bacterial elements appear more slender and granular. This is evident in staining by the method of Fontés or of Much.

Many investigators have naturally been led to study the possibility of increasing the lipase content of the serum of tuberculous subjects either by feeding them with animal fats such as cod liver oil, or by subcutaneously injecting gradually increasing quantities of these same fats, or of other lipoids such as lecithin, or even killed tubercle bacilli. Such experiments, which I have myself repeated on various occasions, have not given encouraging results. One succeeds indeed by graduated injections of monobutyrin or neutral fats, in perceptibly increasing the lipase content of the serum, but normal or already tuberculous animals treated with oils of animal origin (neat's-foot oil or cod liver oil, for example), or with lecithin or other lipoids, do not show any greater resistance to tuberculous infection than do the controls. As for injections of killed tubercle bacilli, they cause a diminution of lipase action and an increase of antitryptic action on the part of the serum of guinea pigs and rabbits (Nina Kotschneff).[6]

Other attempts have been made in adding emulsions of beeswax or emulsions of waxes extracted from tubercle bacilli by cold or boiling xylol to fats. With C. Guérin,[7] at the Pasteur Institute at Lille, I made a number of experiments with these products upon cattle. The result was again a failure.

In none of our animals treated with lipoids, whether preventively or therapeutically, did we observe any immunity, even partial, to experimental infections.

Nevertheless the larvae of certain insects such as the *Achrae grisella*, the *Bombyx molitor*, etc., are capable of digesting waxes. Metchnikoff in his laboratory had Metalnikov[8] study from this point of view the caterpillar of a species of moth which frequently infests beehives. This caterpillar of *Galleria melonella* cannot be fed with tubercle bacilli in place of the wax which is its normal food. According to Metalnikov, the larva nevertheless destroys the bacilli with

[6] Biochem. Ztschr., 1913, **55**, 481.

[7] Ann. de l'Inst. Pasteur, 1914, **28**, 329.

[8] Arch. des sci. biol. de Petrograd, 1906, **12**, 300; 1907, **13**, 169:—Compt. rend. Soc. de biol., 1914, **76**, 95:—Ztschr. f. Immunitätsforsch., 1914, **22**, 235.

extreme rapidity when the latter are inoculated into its tissues, and the destruction takes place within the leucocytes where the bacilli are not recovered except in the form of a brown pigment. Later researches of W. V. Konstantinowitsch[9] (of Kiew) showed, however, that after 5 to 10 days the bacilli were still intact and their virulence unmodified. Moreover, it is impossible to demonstrate lytic action upon tuberculous waxes *in vitro*, either on the part of the blood of the caterpillar or of the protoplasm of its leucocytes. Nor is it possible to obtain, through repeated injections of emulsion of tuberculous waxes or of beeswax, the production of a wax lysin (*cerolysin*) in the serum of warm-blooded animals. All attempts to this end by various experimenters including myself have been fruitless and the sole favorable results announced by Deycke-Pascha and Reschid-Bey[10] could never be confirmed.

C. COAGULINS

The *coagulins*, by producing a sort of condensation of the bacillary protoplasm, modify the physical and chemical state of the bacteria so that they become susceptible to agglomeration either *in vivo*, in the body fluids, or even *in vitro*, as we see in the phenomenon of *agglutination*.

The agglomeration *in vivo* results in the clumping of the bacilli in masses among the nuclear débris of the dead phagocytes; and the intervention, with later degeneration, of the large mononuclear macrophages to form giant cells characteristic of tubercle formation.

The agglomeration *in vitro*, which can be accomplished in a watch crystal or test tube by bringing together a suspension of bacilli and the serum of a tuberculous subject, is evidence that the serum contains coagulins free in the albumins of the blood of the patient, and the amount of these coagulins is shown by the quantity of serum which must be added to a given quantity of bacilli in order to bring about complete agglutination of the latter.

The phenomenon of agglutination *in vitro* may be utilized for the diagnosis and even, in a certain measure, for the prognosis of tuberculous infection. Later, in this same chapter, we shall study the proposed practical applications of this reaction.

[9] Ztschr. f. Hyg., 1909, **63**, 224.

[10] Deutsch. med. Wchnschr., 1907, **33**, 89.

D. LYSINS

As opposed to the coagulins, the lysins, as Maurice Nicolle[11] has clearly shown, are agents of decondensation "which attack the bacterial cells more or less violently and from them liberate poisons which may be called *true endotoxins.*" They also act upon the toxins, and in the special case of tuberculous infection, upon the tuberculins.

To the *lytic* action of the body fluids of tuberculous subjects upon the bacillary endotoxins and upon the tuberculins, are probably due the local or general tuberculin reactions and also those produced in these same subjects by the injection of dead bacilli, whether entire, or freed of their waxy fatty material by appropriate solvents (ether, chloroform, xylol).

Since the specific lysin does not exist in the body of healthy individuals, it will be understood why tuberculin, bacillary extracts and dead bacilli, have no reaction effect in such persons.

Lysins contained in the serum of tuberculous individuals cannot be demonstrated *in vitro* as can the coagulins. They do not appreciably modify either the external form nor the physical properties of the tubercle bacillus. Nor do they dissolve its framework of chitin, wax and fats, which apparently is left untouched. They attack only the products of secretion and the protoplasmic juices, when able to reach them through the ectoplasm.

And yet certain investigators believe that they have observed, both *in vivo* and *in vitro*, a true lysis, at least partial, of the bacilli treated under certain conditions by more or less prolonged contact with the blood or other humors of tuberculous animals.

Thus, according to Deycke and Much,[12] Much and Leschke,[13] R. Kraus and G. Hofer,[14] and Wilfred H. Manwaring and J. Bronfenbrenner,[15] if bacilli are injected into the peritoneal cavity of tuberculous or artificially immunized guinea pigs, the bacilli quickly disappear from the peritoneal exudate and in the fluid are found atypical granular forms, not stainable by Ziehl, but resembling fragments of bacteria.

[11] Ann. de l'Inst. Pasteur, 1908, **22**, 132; 237.

[12] Beitr. z. klin. d. Tuberk., 1910, **15**, 277.

[13] Ibid., 1911, **20**, 405.

[14] Deutsch. med. Wchnschr., 1912, **38**, 1227:—Wien. klin. Wchnschr., 1912, **25**, 1111.

[15] J. Exper. Med., 1913, **18**, 601.

In this manner too Karwacki (of Warsaw), Coggia Figari and Marzagalli and the pupils of Maragliano (of Genoa) attribute to the antituberculous serum prepared by this scientist the property of dissolving bacilli *in vitro* after prolonged contact in the incubator. The bacterial elements are said to lose their staining reaction and to become granular, and rabbits do not contract tuberculosis when inoculated with them into the anterior eye chamber.

I tried to repeat these experiments, not only with the "bacteriolysin" which Professor Maragliano was good enough to send me, but also with other sera to which bacteriolytic properties were attributed by the originators (the sera of Vallée, and of Rappin); and with my collaborators C. Guérin and R. Letulle I tested the ideas advanced by Deycke and Much, Kraus and Hofer, then by E. Rist, Léon Kindberg and J. Rolland[16] on intraperitoneal bacteriolysis in tuberculous guinea pigs. I was never able, however, to prove the actual lysis of a bacillus *in vitro,* and was forced to the conclusion that the observers who thought that they had seen it produced under their eyes must have been misled by the fact that they were using strongly agglutinating sera. As a matter of fact, the bacterial elements adhere to one another in clumps under the influence of these sera, or in the peritoneum of tuberculous guinea pigs, but they are in no wise dissolved. Their fatty-waxy ectoplasm protects them against lytic action.

It is perfectly true that if bacilli be injected for comparison into the peritoneum of normal guinea pigs and of those previously tuberculized by the subcutaneous path (subaxillary by preference, as did Manwaring and Bronfenbrenner, E. Rist, Léon Kindberg and J. Rolland), or intraperitoneally (Et Burnet),[17] and if the peritoneal fluid be withdrawn with a fine pipette at intervals varying from 15 minutes to 24 hours, one finds that, in the tuberculous guinea pigs, the bacterial cells, phagocytized in lesser number by the polynuclears than in the normal guinea pigs, are present in small dense pearl-like masses. If the animals are sacrificed at varying intervals after the injection, one finds, in the normal guinea pigs, small masses disseminated almost everywhere over the surface of the peritoneum and there forming little nodules, while in the tuberculous guinea pigs these masses are collected in balls of variable size and located almost exclusively upon the omentum. But within these nodules the bacilli are always intact. There is no bacteriolysis.

[16] Ann. de méd., 1914, **1**, 310; 375.
[17] Ann. de l'Inst. Pasteur, 1915, **29**, 119.

On the other hand, if bacilli and variable quantities of fresh serum from tuberculous subjects or hyperimmune animals are put into the incubator at 37°C. for periods of time varying from 24 hours to 24 days, and if the mixtures are next inoculated into the peritoneum of normal guinea pigs, the latter almost always become more rapidly and intensely tuberculous than do other pigs inoculated with equal quantities of bacilli which have been allowed to macerate for the same periods of time in normal serum deprived of complement by preliminary heating at 58°C. It seems therefore that, *far from destroying the bacilli in vitro, the sera of tuberculous subjects liberate toxic substances from them* which favor infection and intoxication by virtue of the protoplasmic lysins which they contain (*aggressins* of O. Bail).[18] The lysins, however, exert no solvent action upon the waxy fatty content of the bacilli.

According to researches of J. Bartel,[19] and later Neumann and Wittgenstein,[20] of S. Livierato,[21] and of Fontès,[22] the juice of normal mesenteric glands and also, though to a lesser degree, the juice of certain other healthy tissues (the *spleen, liver* and *ovary*), possess the property of attenuating the virulence of tubercle bacilli on prolonged maceration at 37°C., whereas lung juice and the blood are said to be without effect.

It was known, from the early experiments of S. Arloing, that tubercle bacilli isolated from lymphatic glands are generally less virulent than those obtained from tuberculous foci in other organs. But, on more careful study, it is found that glands which contain tuberculous nodules in process of caseation, or actually caseated, constitute always, after being carefully ground up, an excellent material for obtaining cultures and for experimental inoculations, so that it is not possible to attribute any protective or attenuating properties to lymphoid tissue or to demonstrate within it the presence of specific lysins.

From what has been said, it simply appears that the *lysins* play a preponderant rôle in the sensitization of the tuberculous body against reinfections by the bacillus just as against tuberculins and

[18] Wien. klin. Wchnschr., 1904, **17**, 846; 1905, **18**, 211
[19] Ibid., 1905, **18**, 881.
[20] Ibid., 1906, **19**, 858.
[21] Centralbl. f. Bakt., 1910, **54**, 332.
[22] Ibid., 1909, **50**, 78.

protoplasmic bacillary poisons, whereas the *coagulins* should tend on the contrary to fix the bacilli and to agglutinate them in masses—thus favoring the development of the tubercle—which should be looked upon as a symbiotic process of defense.

E. PHENOMENON OF AGGLUTINATION AND ITS PRACTICAL APPLICATIONS

The first systematic researches on the agglutinating power of the serum and different body fluids of tuberculous individuals are those of S. Arloing.[23] They were made by this scientist in 1898 with so-called *homogeneous* cultures, which he obtained by a special technique.

Starting with cultures from various sources upon glycerin potato medium in which the liquid at the bottom of the tube is commencing to be covered with a thin film, the latter is submerged by gentle shaking. As soon as the film reforms, it is again shaken. By repeating this process every day the bacilli become dissociated and evenly suspended. A small quantity is then taken up with a pipette and divided among a series of flat bottom flasks containing 5 or 6 per cent glycerin broth. These are incubated at 38°. Each day they are shaken several times or still better, the flasks are placed upon a shaking apparatus of any sort whatever (dynamo, turbine or hot-air motor). For 4 or 5 days the broth remains perfectly limpid, then a slight sediment appears at the bottom of the flasks and little by little the whole mass becomes opalescent. The culture, replanted several times under the same conditions in fluid medium and shaken, no longer forms bacterial clumps or masses. Examined under the microscope, in hanging drop, the bacilli are seen isolated or in small groups whose elements appear slightly motile. When the shaking process is stopped, the culture finally forms a sediment and the supernatant liquid becomes clear. But the sediment, if reinoculated into a new flat bottom flask containing broth, produces again a homogeneous culture, and if transplanted upon glycerin potato develops in the form of a yellowish-gray coating which is oily and glistening.

In order to determine the agglutinating power of sera, S. Arloing and Paul Courmont recommend the use of cultures in which the

[23] Compt. rend. Acad. des sci., 1898, **126**, 1319; 1398; 1550.

clouding is thoroughly uniform and aged from 4 to 5 weeks.[24] A small quantity is diluted in 50 to 60 volumes of 0.8 per cent sterile physiological salt solution and 5, 10, 20 and again 20 drops respectively of this dilution put into four test tubes. Each tube except the fourth, which serves as a control, receives later one drop of the serum to be studied.

The four tubes are placed upon a rack, inclined at an angle of 45° and left undisturbed for 3 to 5 hours. If the serum possesses agglutinating power, the liquid becomes clear and the bacilli sediment out in fine punctate clumps. If there is complete clearing in the three tubes and none in the control, it may be concluded that the serum agglutinates at 1 in 20. If only the first two tubes are cleared the agglutinating titre is 1:10.

Macroscopic examination of the sediment however is not enough. Microscopic examination must also be made. To that end the tubes are gently shaken; a drop is taken up with a pipette and examined in hanging drop or between slide and cover slip. While, in the control preparation, the bacilli appear isolated or in scanty groups, they are found packed in clumps when they have been in contact with an agglutinating serum.

Buard[25] modified this technique. He prefers to pour the same quantity of dilution of culture, say 15 drops, into four tubes and to add to three of them, 1, 2 and 3 drops of serum—the fourth tube to serve as control.

Instead of tubes, watch crystals may also be conveniently employed and agglutination be observed under the microscope with low magnification.

Furthermore instead of fresh cultures, it is possible to utilize those killed by the addition of 0.25 per cent formol. They are thus preserved agglutinable for at least 15 days.

The agglutination titre usually observed with sera of tuberculous patients varies from 1 in 5 to 1 in 20. It seldom reaches 1 in 30 and exceptionally 1 in 50.

P. Courmont recommends that the agglutinability of the culture be always tested with a standard serum of known agglutinating power. He also insists that the reaction in a given subject is of value only

[24] Compt. rend. Acad. des sci., 1898, **127**, 312; 425:—Rev. de la tuberc., 1904, 2. s., i, 133; 330.

[25] Thèse, Bordeaux, 1900.

above the upper limit of agglutinating power of the serum of normal subjects of the same species and of the same age. In fact normal sera of a large number of animal species agglutinate the tubercle bacillus, as also the typhoid bacillus, the bacillus coli and many other microbes. Horse serum for example agglutinates at 1 in 20, often at 1 in 50, and Arloing has shown that the serum from a normal cow agglutinates at 1 in 5, whereas the serum of a calf does not agglutinate.

One must understand too that the reaction is not strictly specific. Animal serum which is non-agglutinating may become so if certain chemical substances like guaiacol, eucalyptol, etc. are injected under the skin of that animal. On the other hand the sera of certain patients suffering from a variety of infectious diseases (pneumonia, typhoid fever, erysipelas, icterus, grippe) frequently become agglutinating, only to become once more non-agglutinating after convalescence.

A positive reaction moreover is no indication that one is dealing with a progressive tuberculosis, since it is observed in many individuals clinically non-tuberculous. Patients seriously ill often give a negative reaction.

Statistics of Arloing and P. Courmont set the proportion of positive reactions at 87.9 per cent in proven tuberculosis, at 34.6 per cent in suspects, and at 26.8 per cent in supposedly tuberculosis free. In surgical tuberculosis, however, positive reactions are said to occur in 100 per cent of cases.

Authors who have methodically studied sera of healthy subjects report rather different figures. Schraff for example, finds 42.5 per cent of positive reactions, Sabareneau and Salomon, 59 per cent, V. Grysez and Job in young soldiers 40.5 per cent, Massius and L. Béco among the hospital personnel at Liège, 56.7 per cent.

It is very probable that these positive reactions indicate a latent tuberculosis, as do those of tuberculin, so that the agglutinin reaction in serum is of clinical value only when the result is negative.

Various body fluids other than the serum of tuberculous subjects also contain agglutinins. P. Courmont found them almost constantly in pleuritic effusions and was of the opinion that the prognosis of the pleurisy was the more favorable the higher the agglutination titre. They are also present now and then in ascitic fluid and in that of hydarthrosis and of hydrocele. They are not present, on the other hand, in meningeal effusions. L. Karwacki[26] called attention

[26] Compt. rend. Soc. de biol., 1911, **70**, 272.

to their presence in the sputum of phthisical patients where they are said to be particularly abundant.

Sera of children new-born of tuberculous mothers do not, generally speaking, agglutinate the tubercle bacillus (Lagriffoul,[27] Descos).[28] However, when agglutinins are very abundant in the blood of the mother, their presence may be disclosed in the fetal organism, but in much smaller amount.

Cattle were systematically autopsied by S. Arloing after the sero-reaction. The result was positive in 69 of 70 tuberculous animals and uniformly negative in 80 animals free from tuberculosis.

It seems, therefore, that in the course of tuberculous infection the different body fluids acquire the property of agglutinating bacilli in a state of stable suspension such as may be had with the so-called *homogeneous* culture of S. Arloing.

It is, however, not absolutely necessary to have recourse to this type of bacillus, which is profoundly altered in its biological properties, to the extent of having lost almost all of its virulence, at least for cattle. It will be found just as convenient to use other bacilli, either those rendered capable of suspension by culture upon glycerin bile potato (Calmette and Guérin), or bacilli rubbed up in an agate mortar in the presence of either a little beef bile, yolk of egg, a few drops of a water-alcohol lecithin solution or even of alkali.

The agglutinability of tubercle bacilli varies considerably according to their source, whether bovine, avian or human, according to the media on which they have been cultivated, and according to the concentration of the suspension prepared from them.

At the same time the agglutinating power of sera varies greatly with the individual from whom it is derived, the stage of the disease, the temperature at which it is made to act upon the bacilli, and according to the method of preparation of the bacterial suspension.

In every experiment therefore, the above conditions must be specified.

As we shall see further on, in the course of experimental antituberculosis immunization that the agglutinating power of serum increases from the beginning until it reaches a titre considerably above that observed in sick patients. Later it falls, so that *there is no parallelism between agglutinating power and immunity.* With the serum of

[27] Compt. rend. Soc. de biol., 1903, **55**, 1115.

[28] J. de physiol. et de path. gén., 1903, **5**, 127

a horse treated during a period of one year with 5 injections of from 10 to 70 milligrams of virulent human bacilli intravenously, G. Sobernheim[29] succeeded in obtaining agglutination titres varying from 1 in 1,000 to 1 in 5,000. In my experiments with C. Guérin, our sera of hypervaccinated cattle agglutinated in dilutions up to 1 in 12,000.

F. PHENOMENON OF PRECIPITATION.—TUBERCULOUS PRECIPITINS AND PRECIPITIN-DIAGNOSIS

In a communication before the Academy of Sciences in 1909, I showed with L. Massol[30] that when cattle are immunized with intravenous injections of bacilli cultivated upon bile their sera give a precipitate in the presence of various tuberculins.

André Jousset,[31] Vallée and Finzi[32] soon observed the same fact and attempted to utilize these potent precipitating sera for the diagnosis of tuberculosis, mixing, for example (Jousset), 8 drops of a suspected serous fluid with 32 drops of precipitating serum. The results, after one hour in the incubator at 38°, are too irregular for the reaction to be regarded as of practical utility.

Vincent and Combe,[33] at about the same time, were no more successful in trying to apply the precipitin reaction to the diagnosis of tuberculous meningitis. They mix 100 drops of fresh cerebrospinal fluid with one drop of raw tuberculin. After 12 hours incubation at 38° or at 55° there is a very distinct clouding. But this cloud appears also with cerebrospinal fluids from non-tuberculous meningitis, and with those of cerebral syphilis (Vincent, Straus, Teissier) or typhoid fever. However, a negative reaction should enable one to exclude the suspicion of tuberculosis.

Some analogous experiments had already been made by Bonome in 1907.[34] He prepared extracts of human or bovine bacilli or of tuberculous organs and added them to sera of tuberculous individuals. The result was a precipitation so definite that Bonome thought himself able, not only to make the diagnosis, but also to ascertain the human or bovine origin of the disease.

[29] Ztschr. f. Immunitätsforsch., 1910, **5**, 349.
[30] Compt. rend. Acad. des sci., 1909, **149**, 760.
[31] Compt. rend. Soc. de biol., 1909, **67**, 758.
[32] Ibid., 1910, **68**, 127; 259.
[33] Ibid., 1909, **66**, 918; **67**, 765.
[34] Centralbl. f. Bakt., 1907, **43**, 391.

Damman and Stedefelder, then Zwick[35] quickly proved that this technique gave no information which was of value. Szaboky,[36] Stoerck,[37] and Porter,[38] showed further that the reaction here concerned is very often positive with the serum of healthy subjects.

Porter methodically studied the precipitins in the sera of 682 subjects of whom 381 were tuberculous. Among the latter 25 had received tuberculin treatment.

The technique consisted of mixing, in a test tube, equal parts of 1 in 50 bacillary extract, and 1 in 20 serum. The bacillary extract was a filtrate secured by passing a tuberculous suspension through porcelain and supplementing or not with carbolic acid. The mixture was incubated at 37°C for 12 hours.

The author found a positive reaction in:

12 per cent of healthy subjects
35 per cent of cases of incipient tuberculosis
60 per cent of cases chronic tuberculosis
20 per cent of tuberculosis cases which were cachectic or very severely ill.

The sera of the 25 patients who had undergone tuberculin treatment were all positive.

F. Bezançon and Serbonnes[39] carried out some researches along similar lines, using as their precipitable substance a filtrate obtained by passing a suspension of 3 gms. of human tubercle bacilli ground up in 60 cc. of physiological salt solution, through a Chamberland bougie, and sterilizing a half hour at 120°C. They diluted this bacillary extract to 1 in 10 before adding it to the unheated sera. The mixture was left for one hour in the incubator at 37°C. and 12 hours at laboratory temperature.

The results obtained under these conditions have led Bezançon and Serbonnes, and also Milhit,[40] to conclude that the precipitin reaction has no diagnostic value. It appears to them devoid of all specificity, since sera from pneumonia and typhoid cases, for example, give a very abundant precipitate. However, up to a certain point,

[35] Ztschr. f. Tuberk., 1909, **14**, 276.
[36] Folia Serol., 1909, **3**, 172.
[37] Wien. klin. Wchnschr., 1909, **22**, 808.
[38] J. Infect. Dis., 1910, **7**, 87.
[39] J. de physiol. et de path. gén., 1909, **11**, 1097.
[40] Rev. de la tuberc., 1910, 2. s., **7**, 208.

it may be regarded, when intense, as an element of favorable prognosis, since it diminishes or disappears in grave cases.

The outcome of my investigations with L. Massol[41] enabled us to establish the following facts which throw light upon the mechanism of the phenomenon:

1. Serum of tuberculous subjects (men, cattle and guinea pigs), whether heated or not, rarely gives a precipitate in the presence of solutions of tuberculin or of broth filtered from cultures of human or bovine bacilli. In one of our sets of experiments, 12 sera of phthisical patients and 5 sera of cattle condemned at the abattoir because of tuberculosis were studied in parallel series with bacillary extract. The precipitation reaction was found positive only once; and that was in a case of tuberculosis in an apparently perfectly healthy ox.

2. The serum of cattle or of horses hypervaccinated against bovine or human bacilli, and at times the serum of tuberculous subjects as well, furnish frequently a very definite precipitate when they are diluted with 5 volumes of distilled water. This precipitate may be separated by centrifugation: 100 grams of two of our sera yielded respectively 908 and 913 milligrams. It is insoluble in physiological salt solution. Under like conditions serum of normal cattle gives an imponderable precipitate.

The same precipitation reaction with *distilled water* is often to be observed with sera of subjects suffering from various infectious diseases (typhoid fever, pneumonia, typhus fever).

It is therefore not specific, but is evidence apparently of the setting free of a more or less large quantity of *globulins.*

3. When to the serum of an animal hypervaccinated against the bovine bacillus variable quantities of different tuberculins (aqueous bacillary extract, tuberculin of Koch or filtered broth from culture) are added, a precipitate is constantly formed. The quantity of this precipitate may be measured for each serum against a single tuberculin. Thus with 2 of our sera, 1 cc. sufficed to detect, after 1 hour at 37°C., 0.05 mgm. of tuberculin (bacillary extract). This same quantity of serum exhausted 5 mgms. of tuberculin, that is to say, after centrifugation of the precipitate, a further addition of tuberculin failed to yield a precipitate. The precipitate obtained was insoluble in pure water or physiological salt solution; it was redissolved in water weakly acidulated with hydrochloric acid or rendered alkaline with soda; it was precipitated anew when the soda was neutralized with

[41] Compt. rend. Acad. des sci., 1910, **151**, 285.

acetic acid or carbonic acid. In weakly acid medium it coagulated on heating at 68°C.

This precipitate is not made up of tuberculin since, after several washings and successive centrifugations, it is found inactive in tuberculous subjects, either on subcutaneous injection or by cuti- or ophthalmo-reaction, or even by intracerebral inoculation into tuberculous guinea pigs.

Nor is it constituted of sensitized tuberculin (in the sense of Besredka's vaccines, and contrary to the idea expressed by Vallèe), since in doses of precipitate corresponding to the initial tuberculin it does not absorb complement, and does not give the fixation reaction of Bordet-Gengou.

On the other hand, *the same serum treated with the quantity of tuberculin capable of producing the maximum precipitate*, or by smaller quantities, and *from which the precipitate has been separated by centrifugation, contains approximately all the original tuberculin.* With dilutions of this serum rid of precipitate, the same tuberculin reactions (subcutaneous, cuti or ophthalmic, intracerebral toxicity) are obtained as with solutions of tuberculin of the same titer. Therefore it contains no *antituberculin.*

4. Sera of animals hyperimmunized against tuberculosis also give reactions, although irregularly, with extracts of pseudo-tubercle bacilli (acid-fast bacilli of grass, of dung, etc.) and at times with mallein. When they precipitate the latter substance, the yields are practically the same as those with tuberculin. Sera precipitated by mallein no longer precipitate with tuberculin, and inversely.

As a consequence, one is forced to admit that whether one is dealing with sera of tuberculous subjects or with sera of animals hypervaccinated against bovine or human tuberculosis, *the precipitates formed in mixtures of serum + tuberculin are constituted neither of natural tuberculin nor of sensitized or neutralized tuberculin, since the total quantity of active tuberculin (characterized by tuberculin reactions and by the degree of intracerebral toxicity in the tuberculous guinea pig) remains in the supernatant fluid.*

Although in the light of these results, there is a real and undeniable biological interest in the phenomena of precipitation frequently observed when sera of tuberculous subjects are placed in contact with tuberculin, one is nevertheless obliged to conclude that it is not possible to take advantage of them for the diagnosis of tuberculous infection.

CHAPTER XXXI

REACTIONS OF DEFENSE ON THE PART OF THE BODY AGAINST TUBERCULOUS INFECTION (CONTINUED)

Alexin and Sensibilisatrices or "Antibodies."—Opsonins.—Cytology of Sero-fibrinous Exudates or Effusions.—Cytodiagnosis

A. ALEXIN AND SENSIBILISATRICE.—TITRATION OF ALEXIN IN BLOOD SERUM

It is known that, among the substances of enzymic nature contained in the sera, there are two which play a particularly important rôle in the defense which the body directs against infectious elements: they are the *alexin* and the *sensibilisatrices.*

Alexin, so named by Buchner in 1892—called *cytase* by Metchnikoff and *complement* (or intermediary body) by Ehrlich—exists in the protoplasm of various cells of the body and is particularly abundant in the polynuclear leucocytes which liberate it into the serum after their death. All normal sera contain it in more or less considerable amount. It is destroyed by heating at 55°C. and is therefore *thermolabile.* By itself it is incapable of any solvent or digestive action on bacterial elements or foreign bodies, but it possesses the property of fixing itself to these elements either *in vivo* or *in vitro,* and of *being absorbed* by them like a mordant in dyeing processes (J. Bordet).

The sensibilisatrices or *antibodies* (to which Ehrlich gave the name of *amboceptors*) are excreted into the body fluids, principally the blood, by the leucocytes and by other cells of various organs (spleen, lymphatic glands, bone marrow). They are found in small quantity in normal sera and in much greater abundance in sera of vaccinated subjects. In the latter they present a specific character which is quite manifest, and which is all the more marked when the immunity is conferred and intensified artificially.

They resist heating at 55°C. and are destroyed only above 65°C. Some even survive temperatures still higher. They are therefore *thermostabile.*

These amboceptors, like complement, when by themselves and alone, do not act upon bacterial or foreign elements. But in the presence of complement, either *in vivo*, or when the latter has been previously *fixed in vitro* (or *absorbed*) by bacteria or by foreign cellular elements, they render possible the digestion or dissolution of these latter. Bordet interprets this phenomenon by saying that *the amboceptors exert a sort of mordant action which enables bacteria* or foreign cells *to fix complement as mordant-treated cloth fixes dye.* The sensitized bacteria and cells then become subjected to the dissolving action of complement or to digestion by leucocytes.

We shall return later (*Chapter XXXVII*) to the study of amboceptors to which at present there is a tendency to attribute important functions in the bodily defense against tuberculous infection. But first let us see whether complement enters into this defense.

It is easy to measure the *complement strength* of a serum by determining the minimum quantity of this serum capable of hemolyzing a fixed quantity of red cells in the presence of a suitable amount of hemolytic amboceptor (specific for these red cells). For this determination the following suffice:

1. Blood cells of an animal species (sheep or goat for example) other than that of the serum to be studied. These red cells, derived from defibrinated fresh blood, should be washed by centrifugating several successive times with physiological salt solution and then taken up in suspension in a quantity of salt solution corresponding to 20 times the original volume of blood.

2. A hemolytic serum prepared by inoculating 4 or 5 times, at 6 day intervals, a rabbit for example, with 2 or 3 cc. of a suspension of washed red cells of sheep or goat. Six or eight days after the last injection the rabbit may be bled and its serum is then *hemolytic for the red cells of the sheep or goat.*

This serum should be *inactivated* (that is to say its complement destroyed) by heating a half hour at 55°C. It then contains only the specific amboceptor, which, in this particular case, is anti-sheep or anti-goat *hemolysin.* The test is then carried out as follows:

Into a series of 10 test tubes there is introduced, with a graduated pipette, 1 cc. of a 1 to 20 suspension of washed sheep or goat red cells, and then an equal quantity of inactivated hemolytic serum,

whose hemolytic strength for sheep or goat[1] red cells has been previously titrated.

Then to each tube, except the first which is to serve as control, there are added progressively increasing doses of the complement-containing serum to be studied, the latter having been previously diluted to 1 in 10 with physiological salt solution (8.5 gms. of NaCl per litre).

The second tube will thus receive 0.1 cc. of 1 in 10 dilution of serum (corresponding to 0.01 cc. of the original serum), the third tube 0.2 cc., the others respectively 0.3 cc., 0.4 cc., 0.5 cc., 0.6 cc., 0.7 cc., 0.8 cc., and 0.9 cc. The quantity in all tubes will finally be made up to 3 cc. by adding salt solution, and the tubes placed in the incubator at 37°C. for a half hour. If, at the end of this time, hemolysis is complete in the tubes which received 0.05 cc. of complement (0.5 cc. of the dilution) or more, and is incomplete or nil in all the others, as also in the control which received no complement, the conclusion will be that the complement strength of the serum studied is $\frac{1}{0.05} =$ 20 *units.*

Several workers have shown that the complement content of sera varies according to the animal species and according to certain physiological conditions (digestion, hunger, age, etc.), or pathological states. Goussew[2] was of the opinion that these variations might be utilized for the prognosis of tuberculosis, but A. Jousset and Paraskeropoulos[3] deny their specificity.

In my laboratory, M. Breton, L. Massol[4] and Minet followed the fluctuations in serum complement in one hundred tuberculous individuals at different stages. They found that the complement strength is greater in febrile than in afebrile cases, but that there is no relationship between the progress of the disease, the stage of its development and the complement content of the serum.

[1] If, for example, 0.01 cc. of this serum is the minimum quantity capable of hemolyzing, in 30 minutes, at 37°C., 1 cc. of a suspension of red cells in the presence of a fixed quantity of fresh guinea pig serum (0.025 cc. of complement diluted 1 in 4 in physiological salt solution), one should add 10 times this dose of hemolytic serum to each tube, that is 0.1 cc.

[2] Thèse, Kazan, 1902.

[3] Compt. rend. Soc. de biol., 1909, **67**, 22.

[4] Ibid., 1909, **67**, 580.

It does not seem therefore that the titration of complement in the serum of tuberculous cases can be of any use in the diagnosis and prognosis of the disease.

But we shall see that complement plays an important rôle in the phenomena of *phagocytosis in vitro*, attributed by Wright to other hypothetical ferments to which he has given the name of *opsonins*.

B. OPSONINS.—DETERMINATION OF THE OPSONIC INDEX

Sir Almroth E. Wright and Douglas[5] had observed that certain bacteria (such as the *Staphylococcus aureus*) were not phagocyted by leucocytes *in vitro* except in the presence of normal serum and that the phagocytosis did not take place when the bacteria were placed in the presence of the same leucocytes freed of all serum by a series of successive washings. From this they drew the conclusion that the serum contains certain *bacteriotropic* substances which *prepare* the microörganisms for absorption by the phagocytic elements, and to these substances they gave the name of *opsonins* (from the Greek word opsoneô, I prepare).

It was already known from the experiments of Denys and Leclef[6] that phagocytosis of the streptococcus proceeds with much greater intensity in the presence of the serum of a vaccinated animal, and Metchnikoff[7] had advanced the idea that specific sera owe this property of *exciting the phagocytic activity* to *stimulins* which do not exist in normal sera.

C. Levaditi and Inmam[8] showed that the opsonins of normal sera, which are destroyed, as Wright and Douglas, and then Neufeld and Huhne had already learned, by heating for 10 minutes at 60°C., may be regarded as identical with complement, whereas the opsonins of specific sera, which are thermostabile like the amboceptors, have a complex constitution which brings them nearer to the latter. They induce certain physico-chemical changes in the ectoplasm of the bacterium thereby rendering it more susceptible to ingestion by the leucocytes.

Be that as it may, the phenomenon with which they have to do is an interesting one to study and capable in certain cases of giving use-

[5] Proceed. Roy. Soc., 1904, **74**, 147.

[6] La Cellule, 1895, p. 177.

[7] *L'immunitée dans les maladies infectieuses.* Paris, 1901, Masson & Cie.

[8] Compt. rend. Soc. de biol., 1907, **62**, 683; 725; 817; 869.

ful information. In tuberculous infection in particular, it would seem that the *determination of the opsonic index provided it can be performed and reperformed by the same observer*, may at times throw light upon the prognosis or serve to guide the clinician in the course of tuberculin therapy. Unfortunately it necessitates a delicate and complicated technique which is an obstacle to its use in ordinary practice.

To determine the opsonic index of a given serum one must combine:

1. Leucocytes;
2. A suspension of bacilli properly prepared;
3. The serum to be studied.

These three elements are mixed in equal parts and left in the incubator at 37°C. for fifteen to twenty minutes. Preparations are then made and stained cold with *Ziehl*, afterward with methylene blue. The bacilli contained in a certain number of leucocytes are counted and the average number of bacilli per leucocyte determined. A figure is thus secured which indicates the opsonic power of the serum in question. But since conditions (concentration of the suspension of leucocytes and of the bacillary suspension) unfortunately change from one test to another, the results obtained are comparable and of value only when brought into relation with a constant factor, always the same in all experiments. This constant factor is represented by a normal serum, that of the observer for example. With the opsonic power of this control serum, one compares the opsonic power of the pathological sera to be studied.

The quotient obtained by dividing the figure representing the opsonic power of one of these sera by that representing the opsonic power of the control serum, gives the opsonic index.

1. *Preparation of leucocytes*

The leucocytes commonly employed are either from human blood or from the peritoneal exudate of a guinea pig. The serologist usually uses his own leucocytes; one needs only to prick the dorsal surface of a finger near the nail after having ligated the finger at the base. About 20 drops of blood are collected in a tapering centrifuge tube in which are 10 cc. of the following solution to prevent coagulation:

Distilled water	1000 cc.
Sodium chloride	8.5 grams
Sodium citrate	15.0 grams

At least 8 to 10 parts of this solution are necessary for one part of blood.

The mixture is made by turning the tube several times upside down and back again, the tube being stoppered with the thumb. Too vigorous shaking should be avoided in order not to alter the leucocytes.

After centrifugation the sediment is washed. This is done by drawing off the supernatant fluid with a pipette, adding 10 cc. of physiological salt solution to the tube and mixing it with the sediment by turning the tube as before. The whole is centrifuged anew. The operation is repeated three times. All the fluid is then removed. The upper part of the sediment now contains the white cells. In order to separate them from the deeper layers of red cells, the tube is inclined and the leucocytes, which spread themselves upon the surface, are sucked up with a special pipette provided with a rubber teat (Wright pipette), and then put in a short test tube of very small diameter.

Leucocytes may also be obtained by injecting saline solution, broth, etc., into the peritoneum of a guinea pig, thus causing the formation of a peritoneal exudate. One or two hours later, a little of this leucocyte-rich fluid is withdrawn from the guinea pig's abdomen by means of a curved finely tapered pipette, the animal being held belly downward by an assistant. The exudate is collected in citrate solution and treated as described above for the blood.

2. Preparation of the bacillus suspension

A young 4 to 5 weeks old culture on glycerin broth is taken. After being sterilized in the autoclave at 100°C. for 20 minutes, it is poured upon a paper filter and washed with physiological salt solution until the latter flows clear. A small quantity of bacilli (about 1 centigram) is then removed from the filter, put into an agate mortar and suspended with constant gentle rubbing while physiological salt solution is being added drop by drop with a fine pipette. Thus there is obtained a milky suspension which is rendered still more homogeneous by shaking in a sterile flask with glass beads. It is then centrifugated (for a few minutes only) to eliminate the clumps.

This homogeneous suspension is diluted with hypertonic salt solution (1.5 per cent is the most favorable concentration) until a definite concentration of bacilli is obtained as judged by the opales-

cence, which should be very slight. For this about 50 cc. of salt solution per centigram of bacilli are necessary.

The suspension thus prepared can be sterilized at 105°C. and preserved in sealed tubes which must be carefully shaken before each test in order to make the suspension of bacilli as uniform as possible.

3. Preparation of serum to be studied

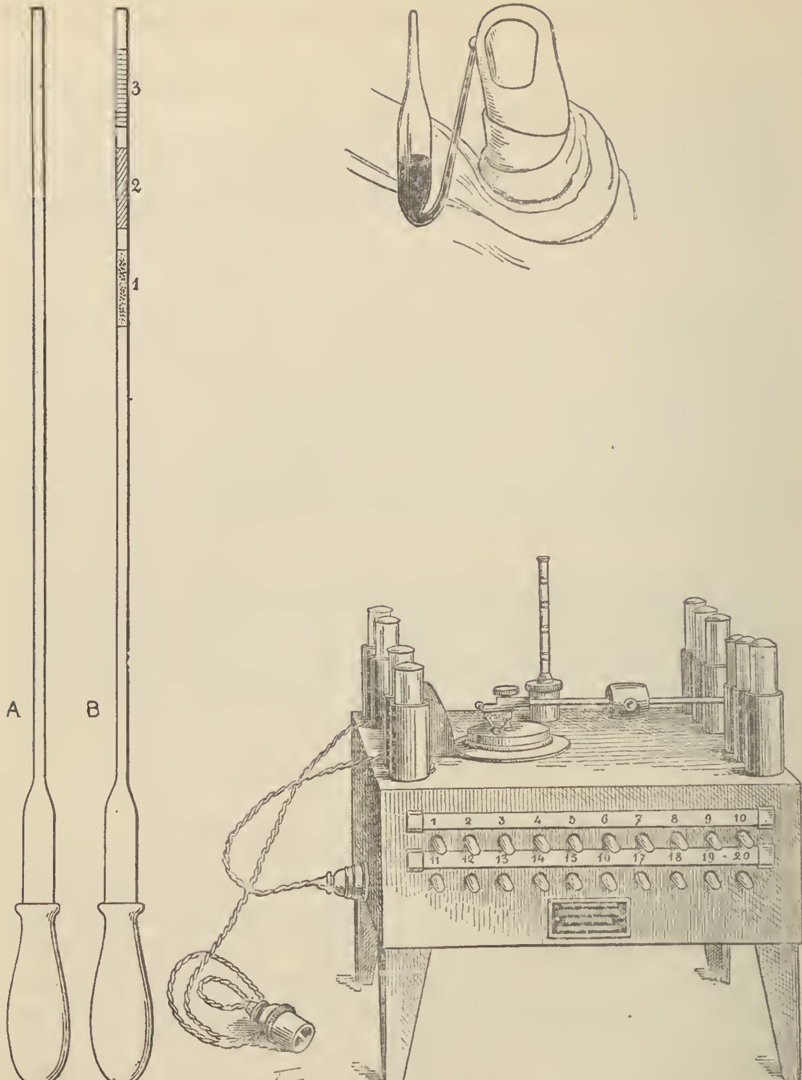
Human blood is obtained by pricking the back of the thumb as already described. The blood is drawn up by capillarity into a small tube with drawn out extremities, one of which has been turned back (Wright capsule). A few drops are sufficient. The serum is left to separate from the clot for 2 or 3 hours and the tube broken at the time of using. In laboratory animals (rabbits, guinea pigs) and also in cattle, the blood is obtained by pricking one of the visible ear veins.

The serum should be clear and perfectly *free from red cells,* since the presence of the latter completely falsifies the result (A. Fleming). It may be preserved in the ice box for 6 to 7 days, which makes it possible to use at one time all of the sera collected during the whole of the week, whether from the same subject or from different individuals.

4. Technique of the reaction

The three necessary elements, *leucocytes, suspension of bacilli* and *serum,* are now ready. A certain number of capillary pipettes are prepared. It is convenient and economical to use for this purpose segments of glass tubing 10 to 12 centimeters long, like those used to make pipettes in the laboratories. They are drawn out at the center, care being taken that the thinned portion is of uniform diameter. The drawn out portion is then divided in the middle and there are obtained two pipettes of equal caliber.

At a distance of 2 cms. from the open tip a pencil mark is made. A special rubber teat serving to aspirate the material is adapted to the pipette. A column of the bacillary suspension is drawn up to the pencil mark, and a bubble of air is next allowed to enter. A like quantity of leucocyte suspension is next drawn in and again a bubble of air is allowed to enter. Finally there is aspirated an equal quantity of serum (*fig. 26*). The whole is then expelled upon a glass slide and mixed by alternately aspirating and expelling, the pipette



2 3

FIG. 26. DETERMINATION OF OPSONIC INDEX BY THE METHOD OF SIR A. WRIGHT

1. Technique for obtaining blood from the finger.

2. Wright pipettes for determining the opsonic index. *A*, pipette ready for use. *B*, pipette containing (1) suspension of bacilli, (2) suspension of leucocytes, (3) serum to be studied.

3. Hearson incubator for the study of opsonins.

being carefully held as vertical as possible. The entire mixture is again drawn into the pipette, the tip of the latter is sealed in a Bunsen flame, and the whole is left in the incubator at 37°C. for 20 minutes.[9]

On removal from the incubator, the tip of the pipette is broken; the contents are blown out upon a clean glass slide and after being mixed anew are spread with the ground edge of a slide.

The preparations are dried for several minutes in the incubator, fixed with absolute alcohol and stained cold for 2 hours at 37°C.

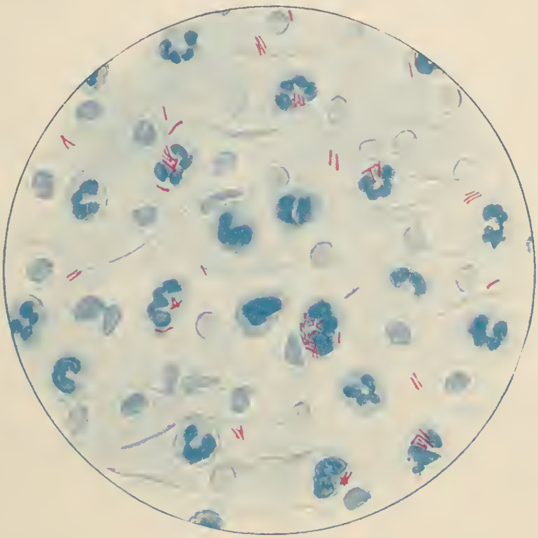


Fig. 27. Phagocytosis of Tubercle Bacilli by Polynuclear Leucocytes
Imm. $\frac{1}{18}$, oc. comp. 6, Reichert

in a solution of *Ziehl* containing only 3 per cent carbolic acid. They are next decolorized with acetic alcohol, washed in water and counterstained for 30 seconds with methylene blue or hematoxylin.

The preparation, having been washed and dried, is ready for microscopic examination. One hundred polynuclear leucocytes are examined and the number of bacilli contained in each one is noted,

[9] A special small incubator has been built for this purpose by the firm of Hearson of London (235 Regent Street), on Wright's specifications (see fig. 26).

zero being put down for each leucocyte showing no phagocyted bacteria (*fig. 27*).

If the bacillary emulsion has not been prepared in proper dilution, phagocytosis is either too abundant or else there is not enough, and a source of error is introduced. The test must then be repeated in an effort to obtain a suspension which gives an average of 2 to 4 bacilli per leucocyte in the presence of serum from a normal subject.

In the counting, clumps of bacilli should be disregarded. Only thoroughly distinct and well stained leucocytes should be noted.

Certain polynuclears contain so many bacilli that it is impossible to count them. In such case Wright and his pupils have adopted the figure 9 invariably.

The *opsonic power* or the *phagocytic quotient* of each serum is fixed by dividing the number of bacteria counted in 100 polynuclears by the figure 100. The opsonic index in then determined by dividing the opsonic power of the pathologic or suspected serum by that of a control serum which is used throughout the series of experiments.

Let us take the control serum T whose opsonic power is, for example, determined thus:

$$\frac{200 \text{ } bacilli}{100 \text{ } polynuclears} = 2$$

and the sera A and B to be tested. If we have:

$$\text{Opsonic power of A: } \frac{400 \text{ } bacilli}{100 \text{ } polynuclears} = 4$$

$$\text{Opsonic power of B: } \frac{100 \text{ } bacilli}{100 \text{ } polynuclears} = 1$$

The opsonic indices will be:

$$\text{For serum A: } \frac{\textit{Opsonic power of } \text{A}}{\textit{Opsonic power of } \text{T}} = \frac{4}{2} = 2$$

$$\text{For serum B: } \frac{\textit{Opsonic power of } \text{B}}{\textit{Opsonic power of } \text{T}} = \frac{1}{2} = 0.5$$

C. CLINICAL VALUE OF THE OPSONIC INDEX

The opsonic index of normal sera for tubercle bacilli varies according to Wright between 0.80 and 1.20; according to Bulloch it is 0.96.

In latent tuberculoses and in the so-called surgical forms (in bones, joints and glands), the index is said to be always below the normal, without however being very low: 0.4 to 0.8 according to Wright and Douglas. In 150 cases of lupus, Bulloch notes an average index of 0.75. Extreme figures which he finds are 0.2 and 1.4 with 75 per cent of the cases below 0.8.

In pulmonary tuberculosis the index shows less uniformity: 0.3 and 1.8 are the extremes found by Wright. Those of Urwick go as high as 2.6. In acute or subacute forms and in bacillemias the opsonic index figures show considerable divergence.

A single index taken separately has in itself no meaning. It is only the study of the *opsonic curve* which is of interest, as has been demonstrated in the clinical studies of Baldwin[10] at Saranac Lake, of Bradshaw and Glynn, of Jousset,[11] of Levaditi,[12] of Bulloch, of Strubell, of Wolff and Reiter,[13] and of Szaboky.

The opsonic index of tuberculous patients, instead of remaining between 0.8 and 1.20 as in healthy subjects, goes beyond these limits. A curve constantly a little below the average is said to signify a chronic or stationary localized tuberculosis. A high curve is said to indicate an arrest in the progress of the disease or the healing of a former infection. Finally a fluctuating curve is said to be a sign of very great value as revealing a tuberculosis *in evolution*, the activity of which can be judged by the amplitude of the oscillations. A constantly very low index should have the same significance.

In the opinion of Wright, a curve without oscillations, a *stable* index, no matter what the figure, is of good prognosis; it indicates an arrest in the forward march of the tuberculosis, a tendency to localization. A variable index, alternately high and low, is on the contrary of bad prognosis.

Wright applied the study of the opsonic curve to the control of tuberculin therapy. Every curve falls (*negative phase*) following the injection of tuberculin. It rises above the unit only after a certain time (*positive phase*). It is during this period that it is advantageous to maintain and fortify, by a further injection, the improvement made. The index serves to indicate the moment when injection will benefit the case.

[10] Proc. Path. Soc. Philadelphia, 1906/07, **9**, 163.

[11] Bull. méd., 1907, **21**, 425; 439.

[12] Presse méd., 1907, ii, 553; 569.

[13] Deutsch. med. Wchnschr., 1909, **35**, 1177.

The effect of tuberculin upon the opsonic index of tuberculous subjects has been repeatedly determined and shows *in vivo* as well as *in vitro* the very definite lowering effect of tuberculin upon the opsonic power of the sera. In my laboratory Manaud proved that 1 per cent of tuberculin mixed with equal parts of serum, had, after one hour in the incubator at 37°C., extracted from the serum both its opsonic and complementing properties.[14]

On the supposition that variations in the opsonizing properties of the sera of tuberculous subjects are perhaps dependent upon the presence of a greater or lesser quantity of tuberculin in the body fluids, I performed with the collaboration of M. Breton and G. Petit,[15] the two following experiments whose results confirmed my hypothesis:

1. Into the peritoneum of a series of normal guinea pigs were injected 5 cc. of physiological salt solution in which were dissolved doses of precipitated tuberculin varying from 1 to 5 mgms. The control guinea pigs at the same time were injected with 5 cc. of physiological salt solution without tuberculin.

Three hours later there was injected into the peritoneum of both the control and tuberculous animals 1 cc. of a suspension of bovine tubercle bacilli previously allowed to settle for 3 hours in the ice box to avoid the presence of clumps.

After a half hour a little exudate was withdrawn by puncture with a pipette, and spread upon slides. The latter were dried in the incubator and fixed for 5 seconds in osmic acid vapor, stained 5 minutes cold with *Ziehl* fuchsin, decolorized with chlorhydrate of anilin and alcohol and counterstained with dilute thionin.

In the control pigs, which were killed soon after and recognized as having no tuberculous lesions, it was found that 100 leucocytes phagocyted an average of 7.3 bacilli. The normal opsonizing power under these conditions is therefore, for each leucocyte, about 0.073.

In the tuberculin treated guinea pigs the opsonizing power was:

For 1 mgm. of tuberculin	0.29
For 2 mgms. of tuberculin	0.24
For 5 mgms. of tuberculin	0.21
For 1 cgm. of tuberculin	0.05
For 2 cgms. of tuberculin	0.05
For 5 cgms. of tuberculin	0.05

[14] Compt. rend. Soc. de biol., 1909, **66**, 563.

[15] Ibid., 1907, **63**, 324.

2. Other normal guinea pigs were injected subcutaneously with a single dose of 2 mgms. of tuberculin. Two hours later a peritoneal exudate was induced in them, as well as in the controls which had received no tuberculin, by injecting 5 cc. of physiological salt solution intraperitoneally.

In the control guinea pigs the phagocytic index of the exudate after a half hour was 0.07.

In those which received tuberculin subcutaneously 12 hours beforehand, it was 0.14.

3. The same experiments were repeated with guinea pigs which received intraperitoneally three separate injections of 1 milligram of tuberculin at twelve day intervals. Twelve days after the third injection, these animals, and the controls at the same time, were injected intraperitoneally with bacilli suspended in 5 cc. of physiological salt solution. After a half hour, part of the exudate was withdrawn.

The phagocytic index in the controls varied between 0.7 and 0.8. In the tuberculin treated animals it was 0.38, 0.40, 0.43 and 0.52.

It is seen therefore that tuberculin introduced either in small single doses or in small *repeated* and *spaced* doses, into the peritoneum or under the skin very manifestly increases the phagocytic power of the leucocytes for the tubercle bacillus. On the other hand the single or repeated injection of large doses of tuberculin reduces it.

The progress of the tuberculosis was however neither hastened nor retarded in any case; generally in the tuberculin treated animals the disease assumed the pleuro-peritoneal type, but death ensued after practically the same interval as in the controls (25 to 42 days).

These experiments do not in any way demonstrate that determination of the phagocytic power is incapable of giving useful information as to the prognosis of tuberculous infections. They only prove that quantitative variations of this phagocytic power are dependent upon the quantity of tuberculin already fixed by the leucocytes or in circulation in the body fluids.

According to certain authors there are several infectious diseases which have great influence upon index variations in the tuberculous. Thus Milhit found the index much lowered for the tubercle bacillus in pertussis, measles, scarlatina and chicken-pox; much raised, on the contrary, in typhoid fever and erysipelas.

At the veterinary school of Dresden, Strubell and Felber[16] studied the variations of the opsonic index in healthy and in tuberculous cattle. For the former they found that this index varies from 0.9 to 1.10 in 87.8 per cent of animals against the human bacillus, and in 71.1 per cent with the bovine bacillus.

For tuberculous cattle, the index was below the normal in 83.3 per cent with the human bacillus, and in 57.8 per cent against the bovine bacillus. Of the tuberculous cattle only 34.3 per cent gave an index above the average for healthy animals.

Non-heated sera of cattle artificially infected with human type bacilli had a normal index in 50.7 per cent of cases with the human bacillus; in 45.2 per cent against the bovine bacillus. The index was below normal in 45.8 per cent of cases against the human bacillus and in 52 per cent with the bovine bacillus.

Sera taken from these same animals and *inactivated by heat*, had an index above 0.30 in 38.3 per cent of animals with the human bacillus and in only 6.8 per cent with the bovine bacillus.

Pochin[17] had already undertaken some analogous experiments under the direction of Wright, utilizing 10 sera of normal cattle, 10 sera of adult men and 10 sera of normal children. His bacillary suspensions were so prepared that scarcely a single bacillus was found phagocyted per leucocyte. For the sera of the cattle the opsonic index obtained was 2.069 with the human bacillus and 1.115 with the bovine bacillus. For the sera of the adult men it was 0.863 with the human bacillus and 2.212 with the bovine bacillus; and for the sera of the children, with the same bacilli respectively the indices were 0.889 and 2.862.

So it would seem that the measure of the opsonic index might serve to evaluate the natural resistance of the body to bacilli of bovine or human origin, since the difference in the reaction of the same serum with each of these appears so great.

Since later experiments conducted by F. Ungermann[18] have questioned the facts announced by Pochin, there should be a new control of the latter with a larger number of sera from various sources. And in any event, from the point of view of the diagnosis of tuberculous infection, the information derived from the opsonic index is of but little value.

[16] Centralbl. f. Bakt., 1910, **54**, 44.
[17] Lancet, 1909, p. 713.
[18] Arb. a. d. k. Gsndhtsamte, 1910, **34**, 286.

D. CYTOLOGY OF SERO-FIBRINOUS EXUDATES OR EFFUSIONS IN TUBERCULOSIS.—CYTO-DIAGNOSTIC METHODS

Cellular reactions brought about by tuberculous infection take the form of certain disturbances in the function of the hematopoietic organs, and these may be disclosed by a study of the cellular elements contained in sero-fibrinous exudates or effusions.

It is some time since Ehrlich showed the antagonism which exists between any infectious process and the eosinophile; and Roger and Josué,[19] and later Hobbs,[20] called attention to the fact, that, while in normal individuals the serous fluid obtained by the application of a vesicant gives an almost constant cell formula (25.6 per cent of eosinophiles, 65 per cent of neutrophiles, 1 per cent of large mononuclears and 8 per cent of small mononuclears), in tuberculous individuals there are either no eosinophiles, or almost none, and hydropic cells are frequently encountered.

To F. Widal and his collaborators Sicard and Ravaud[21] belongs the credit of having laid down exact rules for the cytologic interpretation of exudates. The technique is simple:

About 20 cc. of fluid are withdrawn aseptically, by puncture or trocar, from the pleura, from a joint, from the peritoneum, or by lumbar puncture. If one is dealing with a fibrinous exudate (pleuritic for example) it is necessary first to defibrinate the fluid in a sterile flask with glass beads. If the material is cerebrospinal fluid, which contains no fibrin, it is introduced directly into a tube.

The exudate is next centrifuged until perfectly clear; then decanted, leaving only a few drops of fluid in which to break up the sediment. This is done with a drawn out pipette, after which the sediment is aspirated and quickly spread upon slides. The preparations are dried in the incubator at 37°, fixed in absolute alcohol for several minutes and stained for 2 to 3 minutes with carbolated thionin or with a Romanowsky-Giemsa stain (Giemsa blue), next washed in distilled water and dehydrated in absolute alcohol. They are then ready for examination with a magnification of 400 to 600 diameters.

In all pleural effusions of tuberculous nature the cell formula is characterized by a very great predominance of *lymphocytes* (small mononuclears with large basophile nuclei) whose number increases in

[19] Presse méd., 1901, i, 215.
[20] Ibid., 1903, i, 189.
[21] Ibid., 1900, ii, 324.

the course of the disease, while the polynuclear neutrophiles tend to disappear.

This character is so constant that the tuberculous nature of any effusion may be positively asserted when an abundant lymphocytosis is found.

The same applies to pericardial effusions, to joint effusions and to cerebrospinal fluid. As to the last, if lumbar puncture has been made at the beginning of the disease and a high proportion of lymphocytes is found (in certain cases it may exceed 60 to 80 per cent as against 10 to 30 of polynuclears), the existence of a tuberculous meningitis is beyond doubt.

After the incipient phase the *lymphocytes* diminish in number; *polynucleosis* reappears and at times moreover, in favorable cases, a *mononucleosis*.

In effusions which are the result of experimental inoculation, for example in pleurisy or pericarditis in the guinea pig, the *lymphocytosis* is equally manifest. It represents the first line of bodily defense against infection of the serous membranes by the tubercle bacillus. "It must be remembered," state Widal and Ravaut,[22] "that a serous cavity, such as the pleura, is normally lubricated by a fluid having all the characters of lymph; and the mononuclear leucocytes are a distinctive element of the lymph. In pleurisies which, by reason of their nature, develop without provoking an intense reaction and without necessitating the intervention of powerful defensive agents, such as the polynuclears, the question might be raised whether the effusion does not simply represent, under certain special circumstances, an exaggeration of the normal secretion of the serosa; thus in this case, might be explained the preponderance of lymphocytes in the fluid exuded."

[22] Compt. rend. Soc. de biol., 1900, 52, 1118.

CHAPTER XXXII

REACTIONS OF DEFENSE ON THE PART OF THE BODY AGAINST TUBERCULOUS INFECTION (CONTINUED)

The Blood and Its Cellular Elements

The macroscopical characters and chemical composition of the blood are not perceptibly modified by tuberculous infection, but the blood mass, in proportion to the weight and size of the body, is often reduced in gravely affected individuals, who exhibit pallor and more or less emaciation. The pallid skin in these cases results from insufficient blood pressure. According to R. Fenstell,[1] who studied this question in 20 tuberculous cases in different stages, the pressure is found to be lowered in milder forms as well as in the most severe cases, while elevated in progressive pulmonary tuberculosis and beginning tuberculosis of the apices. Results are said to vary, according to the gravity of the disease, and whether estimations are made before or after the noon meal.

Grawitz[2] thinks that the anemia in the tuberculous, particularly that which is manifest when there are neither fever nor abundant sweats, is due to the lymphagogue action of tuberculous toxins, inasmuch as it is found following injections of tuberculin.

The blood of tuberculous cases is at times less dense than normal, but this lowering is common to all cachexias (Lyonnet). Its specific gravity falls to 1.040 or even to 1.032 as against the usual 1.061. The apparent alkalinity increases in the beginning, to diminish in the stage of cavity formation.

In acute forms, the serum has been observed to become *opalescent*.

Variations in the weight of *dry residue* run parallel with those of the red cells (Grawitz, Appelbaum).

The blood of tuberculous individuals is low in minerals. Albert Robin states that the mineral content, which is about 8.39 gms. in normal subjects, oscillates between 7.85 gms. and 6.38 gms. during

[1] Ztschr. f. Tuberk., 1913, 20, 169.

[2] Deutsch. med. Wchnschr., 1893, 19, 1347.

the early period of the disease. The loss is chiefly in sodium salts, phosphates and iron. The proportion of potassium salts is said to be increased. There is some disagreement as to the loss in calcium phosphate (Becquerel and Rodier).

A. RED CELLS.—RESISTANCE TO HEMOLYSIS.—ANEMIA OF THE TUBERCULOUS

The study of cellular changes in the course of tuberculosis is of no very special interest; it affords almost no useful information as to diagnosis or prognosis (Wolff).

Certain investigators, especially J. Camus and Ph. Pagniez,[3] called attention to the hemolyzing properties of products derived from the tubercle bacillus (the ethero-bacilline of Auclair); but A. Dufourt and Gaté[4] conclude from their researches that the dried and ground cultures contain nothing capable of dissolving *in vitro* the red cells of man, sheep or the rabbit.

The tubercle bacillus therefore does not seem to exert any specific action upon the red cells, either by itself or through its secretory products. Only the somewhat prolonged evolution of the disease and the appearance of certain symptoms indicative of more or less grave anatomical lesions in the various organs, are capable of having an effect upon the blood formula by momentarily lowering the number of red cells,—for example following hemoptysis,—or by increasing them in a rather constant manner (*polycythemia of the dyspneic*).

The number of red cells per cubic millimeter of blood is usually little affected. According to V. Noorden it does not fall as a rule more than 20 per cent below the normal figure which in man is about 5,000,000. In one altogether exceptional case, Malassez found however a diminution of 500,000 in one week. In fibrous phthisis some polycythemia is said to be observed as a general thing and clinicians think that a favorable prognosis may be inferred therefrom. But such a conclusion should be accepted with reserve, since loss of fluid on the part of febrile tuberculous cases through their profuse sweats, diarrhea and emaciation must be taken into account.

It has been claimed that sojourn at a high altitude, in a rarefied atmosphere, brought about a beneficial *hyperglobulism* in patients.

[3] Compt. rend. Soc. de biol., 1901, **53**, 915.
[4] Ibid., 1912, **72**, 320.

But Kuss, by experiments upon guinea pigs, has demonstrated that this was simply the result of a more active elimination of water vapor from the lungs, which is equivalent to substituting a dry regime for a normal diet. *It is incorrect therefore to say that mountain climates act by stimulating hematopoiesis.*

According to M. Labbé[5] anemia in the tuberculous may assume three types:

1. The *anemia with pallor* (*ochrodermie*) of cases which are febrile and emaciated, with drawn face, sunken eyes and hollow cheeks, skin closely applied to the bones, and pale or livid mucous membranes. In these subjects there is always an oligemia; the number of red cells, the hemoglobin content, the color index and the arterial tension are all diminished.

2. The *anemia without pallor*, which is observed in most varied forms, caseous or fibrous, with or without fever, in patients with thin, drawn, tired faces, but whose skin and mucous membranes are of normal color or now and then a little cyanotic. These subjects do not look anemic and are not oligemic.

3. The *pallor without anemia*, characterized by marked paleness and some pigmentation, without vascular disturbances, diminution of red cells or lowering of the hemoglobin content.

Marcel Faure-Beaulieu[6] has quite correctly insisted upon the fact that the destructive action of tuberculosis upon the erythroblasts finds its highest expression, although rarely to be sure, in the so-called *pernicious* form of anemia which always assumes a definitely plastic character. In addition to the observation of Malassez already cited, a certain number of others have recently been published (M. Labbé and Agasse-Lafont, Paul Courmont and Dufourt).

Ribadeau-Dumas and Poisol, in particular, studying severe childhood anemias in the course of acute disease, describe a definitely proved case of pernicious anemia of plastic form in a child who died of miliary tuberculosis. L. Tixier, in a case of pulmonary tuberculosis of 42 years, presenting a profuse diarrhea, found a red cell count below one million with a color index below one, without any nucleated red cells, nor poikilocytosis, and no polychromatophilia. It is probable that in such cases the loss of red corpuscles is bound up with the absorption of hemolytic poisons of intestinal origin.

[5] Bull. et mém. Soc. méd. d. hôp. de Par., 1906, 3. s., **23**, 734.

[6] Rev. de la tuberc., 1911, 2. s., **8**, 157.

But it may also be in consequence of cirrhotic lesions of the liver (without tubercles) which are fairly frequently observed. Orth, Bartel and Neumann, Mouisset and Bonnamour[7] have called attention to their importance. Lintvarev[8] likewise insists upon the fact that the spleen in tuberculous cases is always to a certain extent enlarged and hyperplastic (the pulp comes away easily on scraping the cut surface). At times there is an obvious splenomegaly.

Scholz[9] has studied experimentally the effects of recent tuberculous infection upon the blood of cattle, rabbits and guinea pigs. He became convinced that, in a general way, the percentage of neutrophile leucocytes falls, while that of eosinophiles and lymphocytes increases. The average number of red cells diminishes. On the other hand if tuberculous animals are tested with tuberculin the effect upon the blood is the same, regardless of whether the tuberculin is prepared from human or bovine bacilli. There is an increase of leucocytes and a diminution of red corpuscles; the proportion of neutrophile leucocytes however is lower, while that of lymphocytes and eosinophile leucocytes is higher.

It can therefore be stated that, in tuberculous infection as in syphilis, there is an excessive erythrophagia and that the latter manifests itself by a considerable loss of red cells.

Many workers have studied the *resistance of the red corpuscles* of tuberculous subjects to different agents which have the power of disintegrating or dissolving them (hemolysis).

This resistance is usually calculated by the method of Hamburger, modified by F. Widal and Abrami, which makes it possible to determine either the *minimum* resistance, by putting the red cells in a salt solution where only the more fragile begin to lose their hemoglobin, or the *maximum* resistance, by placing them in so weak a solution that all the corpuscles give up their hemoglobin.

Hemoglobin begins to diffuse from human red cells in general (minimum resistance) in solutions containing 0.44 or 0.48 per cent of sodium chloride. Hemolysis is complete (maximum resistance) at about 0.32 per cent. Variations moreover, in tuberculous cases, are only a few hundredths on the *plus side* according to certain authors (Baumholtz and Lang, Gosdsitski, and on the *minus side* according

[7] Rev. de méd., 1904, **24**, 337.
[8] Ann. de l'Inst. Pasteur, 1912, **26**, 51; 138.
[9] Centralbl. f. Bakt., 1912, **65**, 189.

to others (Chanel, Maragliano, Chkliarevitch, Veyrassat). Brulé,[10] Cade, Morel and Roubier[11] assert that no conclusions can be reached from this study since there are numerous outside factors (secondary infections, hemorrhages, etc.) which take away all value from a reaction which normal physiological phenomena (alimentation, excretions, etc.) are capable of influencing.

Léon Bernard and André Cain[12] consider besides that resistance on the part of the red corpuscles is generally normal, and from their investigations it would seem that the hemolytic icterus and anemia of the tuberculous are due probably, not to the tuberculosis itself, but to secondary disturbances in the body.

With solutions of *saponin*, the fragility of the red cells in tuberculosis is most often exaggerated. The cause perhaps is to be ascribed to disturbances of lipoid metabolism. It is known in fact that hemolysis by saponin is dependent upon the relative proportions of cholesterol and lecithin in the cell membrane: hemolysis is the more intense the lower the proportion of cholesterol to lecithin. Chauffard has shown that tuberculosis is characterized, from the point of view of lipoidemia, by a more or less considerable diminution in cholesterol content. The result is that, in the tuberculous, the proportion of cholesterol to lecithin is lowered, thus bringing on a diminution of the cellular resistance to saponin. On the contrary, a decrease in the ratio of cholesterol to fatty acids results in an increase of resistance to hypotonic solutions.

It may therefore be concluded that disturbances of lipoidemia are responsible for the blood alterations and, consequently, for the anemia of the tuberculous (Et. May).[13]

The color index of the blood varies with the evolution of the disease. The early stage of a grave and progressive infection is generally marked by anemia. Grawitz and Ewing, long since, showed the close relationship between incipient tuberculosis and certain states of anemia not yet definitely classified, such as Hodgkin's disease and the pseudo-leukemia described by the Germans. It should be added that similar relationships can be established between tuberculosis

[10] Thèse, Paris, 1909.

[11] Assoc. Française pour l'avancement des sci., Clermont-Ferrand, 1908.

[12] Bull. Soc. d'étude scient. sur la tuberc., 1913, May.

[13] Ibid., 1914, February.

and essential chlorosis which indeed, in the opinion of Landouzy and his pupils, as well as of Hanot and of Pawlinoff, is but a larval form of tuberculosis or a manifestation of a "paratuberculous hérédo-dystrophy." Although the color index in tuberculous cases falls very little below the normal figure taken as a unit

$$\frac{\text{oxyhemoglobin 14 per 100}}{\text{number of red cells 5,000,000}} = \frac{1}{1} = 1$$

and although, on the contrary, the chlorotic state shows a fall more marked, it does not seem that any practically useful deduction can be drawn from this determination.

Let us conclude therefore with Marcel Faure-Beaulieu that "the principal gain from the later investigations as regards the red cells in the tuberculous is an appreciation of the unreliability of the count as commonly performed, since it informs us only as to the quantity of red cells contained in the unit volume of blood and not as to the quantity of red cells contained in the whole of the blood, a knowledge which would be of much more importance."

B. THE LEUCOCYTES.—FORMULA OF ARNETH

Although study of changes in the leucocytes is of no certain diagnostic value, it nevertheless gives prognostic information which is worth consideration and which has been defined by the works of Oelsnitz[14] for the different forms of tuberculosis in children, and by those of Simon and Spillmann,[15] Richard,[16] Bezançon, de Jongh and Serbonnes,[17] H. Schwermann,[18] and others.

We know that in normal subjects the blood contains from 60 to 70 per cent of neutrophile leucocytes, and from 20 to 25 per cent of lymphocytes. Now, in the blood of cases of incipient tuberculosis or in those who are apyretic, the number of neutrophiles is normal or diminished, and that of the lymphocytes increased (*Plate XXIV*).

It must be granted, in a general way, that benign slowly progressing tuberculosis, fibrous phthisis and glandular forms without exacerba-

[14] Thèse, Paris, 1903.
[15] Compt. rend. Soc. de biol., 1906, **61**, 241.
[16] Prov. méd., 1908, **19**, 166; 205.
[17] Arch. de méd. expér., 1910, **22**, 17.
[18] Ztschr. f. Tuberk., 1914, **22**, 20.

tions, are characterized by a *formula of resistance* (Richard) which consists in a moderate leucocytosis not exceeding 10,000 per cubic millimeter (the normal averages about 7,500) and a fairly pronounced lymphocytosis with a slight eosinophilia.

If, on the contrary, the case be one of massive or highly virulent infection, the leucocytic formula becomes the *formula of defense:* hyperleucocytosis, abundant polynucleosis, mononucleosis with constant diminution of eosinophiles and lymphocytes.

Finally, in open tuberculoses arrived at the stage of softening with cavities and in the grave caseous forms, there is found a marked leucocytosis varying from 16 to 20,000 in which the polynuclears make up a proportion of 90 per cent; the lymphocytes and medium sized mononuclears are decreased in number, as well as the large mononuclears which are often found in process of degeneration; eosinophiles are almost entirely lacking or are degenerated and broken up. This is the *formula of defeat* (déchéance).

However theoretical these formulae may appear, they nevertheless reflect fairly correctly the findings which stand out from the major part of the more recent clinical and experimental studies. At the same time they are affected by numerous accidents which may arise in the course of the tuberculosis, for example hemoptysis, more or less abundant expectoration, associated or superadded infections, and also by various forms of treatment, particularly injections of tuberculin. The latter are said to increase the bodily predisposition to polynuclear, lymphocytic and eosinophilic reaction (Etienne, Remy and Boullangier).[19]

According to J. A. Miller and Margaret Reed,[20] in cases becoming slowly worse or undergoing a transient active extension, there is a marked leucocytosis with a predominance of polynuclears and a proportional diminution in the number of small lymphocytes and eosinophiles. The number of large mononuclears meanwhile remains unchanged.

Ch. Madelaine[21] observed that what he calls "inoculations of derivation" have the effect of suppressing the *polynucleosis* and calling forth an early appearance of the phase of *mononucleosis* which is the true phase of defense against tuberculous infection. This he

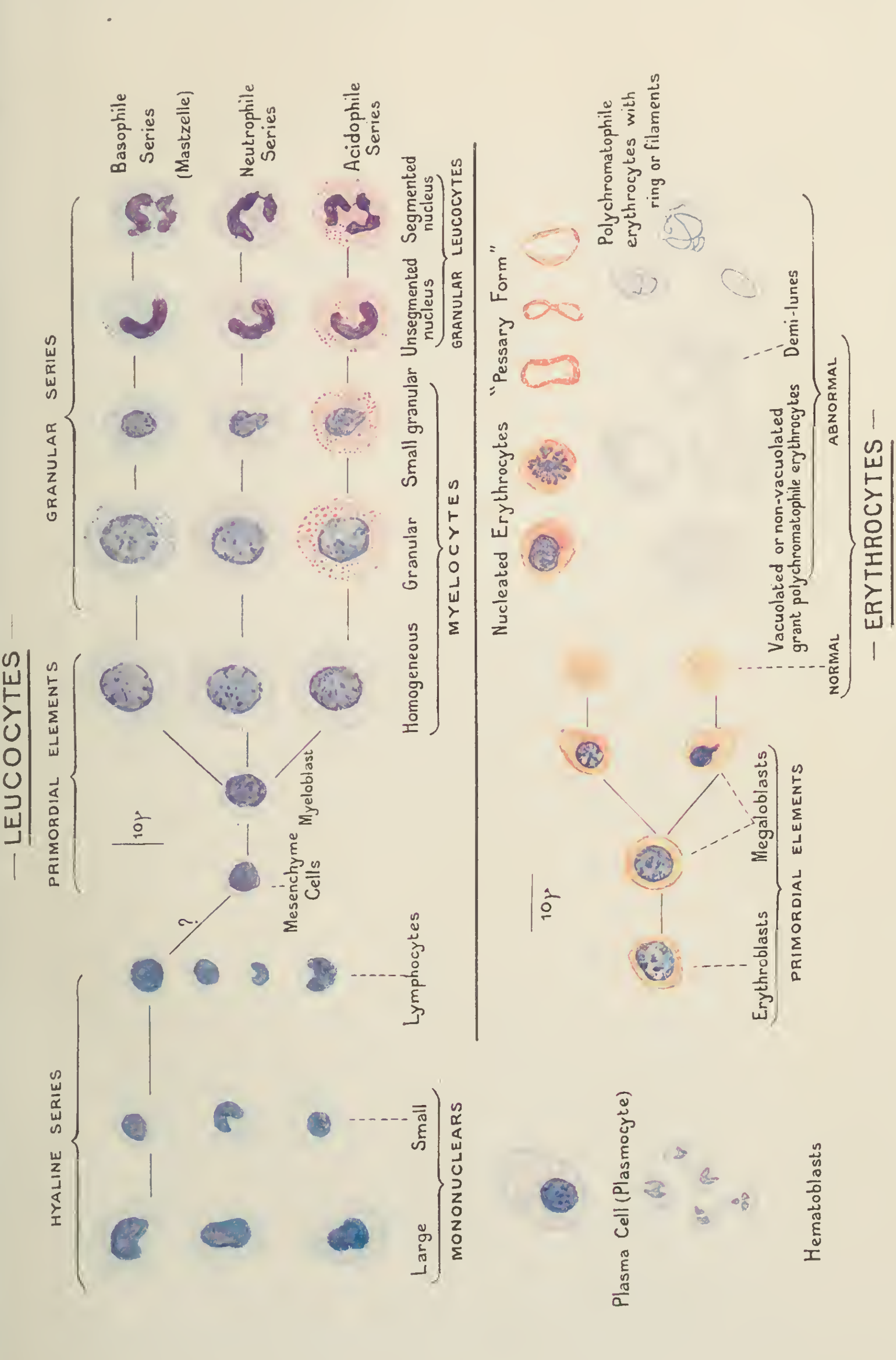
[19] Compt. rend. Soc. de biol., 1909, **66**, 270.

[20] Arch. Intern. Med., 1912, **9**, 609.

[21] Bull. Soc. d'étude scient. sur la tuberc., 1914, May.—Thèse, Paris, 1914.

PLATE XXIV

Human Blood Cells—Normal and Pathological



— LEUCOCYTES —
HYALINE SERIES
PRIMORDIAL ELEMENTS
GRANULAR SERIES
Basophile Series
(Mastzelle)
Neutrophile Series
Acidophile Series
10μ
?
Mesenchyme Cells
Myeloblast
Large
Small
MONONUCLEARS
Lymphocytes
Homogeneous
Granular
Small granular
MYELOCYTES
Unsegmented nucleus
Segmented nucleus
GRANULAR LEUCOCYTES
Plasma Cell (Plasmocyte)
Hematoblasts
Nucleated Erythrocytes
"Pessary Form"
Polychromatophile erythrocytes with ring or filaments
10μ
Erythroblasts
Megaloblasts
PRIMORDIAL ELEMENTS
Vacuolated or non-vacuolated grant polychromatophile erythrocytes
NORMAL
ABNORMAL
Demi-lunes
— ERYTHROCYTES —

ingeniously contrived to observe by injecting a rabbit for example, intravenously, first with *aspergillus* spores killed with heat, then after a half hour or an hour, with a suspension of virulent bovine bacilli.

Polynucleosis in fact is rather an indication of a tuberculosis in process of evolution. Oddo and Monier found it with hemoptyses. Simon and Spillmann, Bezançon, De Jongh and Serbonnes regard it as an important element of unfavorable prognosis.

The study of the leucocytic formula in the course of tuberculosis has gained much from facts recently acquired as to the rôle of leucocytes in natural immunity. Thus Arneth, comparing, from the point of view of the state of progressive ripening of the nucleus, the neutrophiles under normal conditions and during infections, and in particular in infection with the tubercle bacillus, has described what he calls the neutrophilic blood picture (*neutrophiles Blutbild*[22]).

This scientist proposes to divide the neutrophile leucocytes, from myelocyte to polynuclear, according to the number and form of the nuclei, into 5 types, each presenting several distinct varieties:

I. *One nucleus*: (a) round (myelocyte); (b) slightly indented; (c) deeply indented.

II. *Two nuclei:* (a) round; (b) drawn out; more or less looped; (c) one round nucleus, one looped nucleus.

III. *Three nuclei:* (a) round; (b) looped; (c) one round nucleus, two looped nuclei; (d) two round nuclei, one looped nucleus.

IV. *Four nuclei:* (a) round; (b) looped; (c) three nuclei round, one looped; (d) one round, three looped; (e) two round, two looped.

V. *Five nuclei* classified as above.

According to Arneth, under normal circumstances, in man the division is as follows:

5 per cent of neutrophile leucocytes of Type I
35 per cent of neutrophile leucocytes of Type II
41 per cent of neutrophile leucocytes of Type III
17 per cent of neutrophile leucocytes of Type IV
2 per cent of neutrophile leucocytes of Type V

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[22] München. med. Wchnschr., 1905, **52**, 542;—Ztschr. f. Tuberk., 1905, **7**, 309; 405.

For the two best characterized forms of tuberculosis, there are said to be found:

	IN ACUTE MILIARY TUBERCULOSIS	IN CHRONIC PULMONARY TUBERCULOSIS
	per cent	*per cent*
Type I	36	14.0
Type II	56	56.5
Type III	8	24.5
Type IV	0	4.5
Type V	0	0.5

To be sure, quite considerable variations of these different types are often observed, but always in the order indicated above, so that a relationship between the gravity of the lesions and the predominance of neutrophiles of types I and II appears evident. At the same time it is impossible to derive any valuable prognostic information from the study of the formula of Arneth without making a series of examinations in the same subject.

In patients undergoing tuberculin treatment, the formula becomes modified under the influence of the injections and, when it tends to approach the normal, it would seem that the prognosis should be regarded as favorable.

This at least is the conclusion to be drawn from the facts published by Arneth and by other investigators who have confirmed his work, for example, Arnold and Henri Klebs[23] in America, Fernand Arloing and Genty,[24] and E. Brissaud[25] in France. These latter have shown that during the hours preceding death, in pulmonary tuberculosis as well as in other infectious diseases, one observes an almost total destruction of the neutrophiles of groups III, IV and V, while those of group I persist in abundance, their nucleï segmenting scarcely any further and remaining spherical or in the form of an omega.

The technique usually employed for such study of the blood is either that of staining with the triacid stain of Ehrlich, which is allowed to act after fixation of the smears with heat, or, more simply, the procedure of Sabrazès which consists in drying the preparations without heating them and in staining a few minutes in pure medicinal methylene blue in a 1 to 500 solution in distilled water.

[23] Am. J. Med. Sci., 1906, **132**, 538.
[24] J. de physiol. et de path. gén., 1910, **12**, 236.
[25] Thèse, Lyon, 1912.

CHAPTER XXXIII

ELIMINATION OF TUBERCLE BACILLI BY THE VARIOUS EXCRETORY PATHS

A. EXPECTORATION

1. Macroscopic and microscopic characters of products of expectoration

When bacilli appear in the expectoration, it is because there exist in the lungs one or several tubercles in a state of caseous softening whose contents are poured either into the alveoli or into the bronchi communicating with the external air. This discharge may be accidental and momentary or more or less continuous. In the latter case lesions, already multiple and extensive, are evidently present. The abundance and persistence of bacilli in sputa or their small number and the rarity of their appearance constitute therefore important elements in the diagnosis and prognosis of a tuberculous pulmonary infection; but the fact of their being present at a single examination is of no significance except as confirmation of the diagnosis of an open tuberculosis and does not hold except for the moment when the examination is made.

It often happens that old pulmonary lesions or recent progressive extensions are not accompanied by bacilli in the expectoration. Elimination of bacilli through the sputum should therefore not be regarded as the criterion of diagnosis, and above all of early diagnosis. When it does occur, it is because the organic changes are already considerable,—which is not to say however that they are irreparable.

When sputum appears, it shows characters which vary greatly according to the stage, situation and nature of the pulmonary or bronchial lesions which bring about its expulsion. Often at the beginning, in the *fibro-caseous* forms, which are the most common, it retains for weeks or months the mucous or muco-purulent and foamy aspect which is observed in the expectoration of ordinary bronchitis cases. When caseated tubercles begin to rupture the sputa generally

take on a special consistency and a greenish yellow or gray color. They are evacuated in the form of little masses like grains of rice, enclosed in mucus; they are then said to be *nummular* (from *nummularia, in the form of a piece of money*). In this condition they do not adhere to the container and are hard to dissociate. The bacilli are usually numerous and irregularly clumped about the cellular débris (degenerated alveolar or bronchial cells, mono- and polynuclear leucocytes with elongated nuclei) which make up the dense mass.

In *caseous pneumonia*, expectoration in the beginning is like that in acute pneumonia. It is at first rusty, viscous, sticky and translucent, later it becomes puriform. In it is found débris of all sorts of cells (epithelioid, endothelial, mononuclear and polynuclear cells with deformed nuclei) covered with sero-albuminous exudate.

The so-called *galloping phthisis* with multiple broncho-pneumonic foci, and acute miliary tuberculosis are often not accompanied by any expectoration. When the latter is present, it has the same characters as in caseous pneumonia.

The presence of *elastic fibres* in sputum has for a long time been regarded as pathognomonic of tuberculosis. They are evidence of the disintegration of necrotic perialveolar tissue and are consequently particularly abundant at the beginning and during the formation of cavities. In searching for them, the sputum is treated with a little sodium hydroxide solution and centrifugated. The sediment is then stained with the picro-carmine of Orth or according to the technique of Barth which consists in treating the preparation for 24 hours with a mixture of:

Orcein	1 gm.
Water	40 gms.
Alcohol (95 per cent)	80 gms.
Nitric acid	40 drops

The preparation is then washed and differentiated with:

Alcohol (90 per cent)	50 gms.
HCl	1 drop

The elastic fibres stand out distinctly, of a dark brown red color.

F. Bezançon and S. I. de Jongh[1] prefer to use Weigert's special stain called *fuchsélin*. The sediment from the concentrated cen-

[1] *Traité de l'examen des crachats.* Paris, 1913, Masson & Cie.

trifugated sputum is spread upon slides and fixed with absolute alcohol for 5 minutes. The slides are stained with pure fuchsélin for 20 to 30 minutes and then decolorized thoroughly in absolute alcohol. Only the elastic fibres remain stained with violet blue.

The same authors insist quite rightly upon the fact that eosinophile leucocytes are never found in appreciable number in tuberculous sputum, whereas an abundance of them is characteristic of the expectoration in true asthma and emphysematous conditions.

Wolff-Eisner has called attention to the value of the cytological examination of sputum and particularly of that for *lymphocytes* whose abundance, characteristic according to him, appears to be an indication of incipient pulmonary tuberculosis, when tubercle bacilli are not yet to be found. It is said that there are often 32 to 90 lymphocytes per 100 white cells. But just as great a lymphocytosis may exist in the expectoration occurring in the course of other diseases. Arnheim noted it in pertussis and L. Michaelis in certain chronic inflammations of the bronchi.

Macroscopic and microscopic examination of sputum, and even the presence of elastic fibres, do not enable one to make a diagnosis of tuberculosis, unless of course tubercle bacilli are to be found. The same elements and the same appearance may be presented in a large number of diseases (grippe, pneumococcus broncho-pulmonary affections, Malta fever, measles, etc.). As for elastic fibres they are now and then just as abundant in certain pulmonary mycoses (*aspergillosis*).

It is therefore the search for the tubercle bacillus which should chiefly interest the clinician; and it should bear not only upon the constant or intermittent presence of the bacillus, but also upon the number in the preparations, their morphological characters, and their virulence and type, whether human or bovine, if the probable causes of infection are to be determined.

2. Technique of examination of sputum for tubercle bacilli

Examination of the sputum, preferably that taken on awaking in the morning, should be made both of a few opaque purulent particles, and of 5 to 20 cc. of sputum dissolved in antiformin. With a pair of pointed forceps a small purulent fragment the size of a pin head is spread over a thoroughly clean glass slide and the latter quickly covered with another glass slide. The two slides are pressed together

between the fingers and passed one over the other until they are separated. The layer of sputum remaining on each is then as thin and uniform as possible.

With the sputum layer upward the two slides are passed through the flame of a Bunsen burner or alcohol lamp in order to dry and fix. They are afterwards stained by one of the methods already described in Chapter I. The altogether preferable method is that with the staining fluid of Ziehl, which latter has the great advantage of keeping for a long period if protected from light and too rapid evaporation.

I shall simply recall here that this fluid is prepared by triturating 1 gm. of basic fuchsin in a mortar in 10 cc. of absolute alcohol, to which are added 5 gms. of white carbolic acid crystals and then 100 cc. of distilled water in small portions and with continued stirring. The liquid is poured into a flask and, after being allowed to stand at least 24 hours, is filtered.

Slides may be stained either cold or hot. It is very convenient, when several slides are to be stained, to immerse the lower two-thirds in a small glass jar such as I had made by *Leune*[2] and whose small removable cover contains 12 openings. Each of the latter is of the size of a slide. The jar having been filled with the *Ziehl* stain, the whole of the portion of the slide which is covered with the layer of sputum stands vertically in the fluid, and a distinguishing number may be marked upon the part of the slide which remains untouched by the dye.

If there is no hurry the slides may be left in *Ziehl* for 24 hours at laboratory temperature, or for only two hours if the jar is placed in the incubator at 38°C.

If desirable to prepare only one slide at a time and know the result more quickly, a few drops of stain are poured upon the fixed sputum and gently heated at some distance from a Bunsen burner until vapor is emitted. Boiling should be avoided. The stain is then poured off and the slide washed in water and decolorized by adding with a dropper either a 1 in 3 solution of nitric acid, a 1 in 5 solution of sulphuric acid, a 5 per cent solution of lactic acid, a 1 in 3 dilution of acetic acid in 95 per cent alcohol, or again a 2 per cent aqueous solution of chlorhydrate of anilin, the action of which is less intense. The decolorant is allowed to act for some minutes, being renewed from

[2] 28 bis, rue du Cardinal Lemoine, Paris.

time to time according to the thickness of the preparation. In general one or two minutes are sufficient. The slide is next thoroughly washed in 60% alcohol (or in absolute alcohol if chlorhydrate of anilin has been used) until the original red stain has completely disappeared.

The preparation is counterstained with a few drops of a saturated aqueous solution of methylene blue or a 1 per cent aqueous solution of malachite green which is allowed to act for one or two minutes. It is then washed in water under the tap and finally blotted and dried on a warm surface. The slide is now ready for examination with an oil immersion lens, no cover glass being necessary. The bacilli stand out clearly red and are easily found among the other bacterial or cell elements which counterstain blue or green.

The above described method of examination is the one most commonly used and the most simple. But it must be realized that if the bacilli are few in number it does not always reveal them. On that account it is preferable to employ, along with the preceding method and indifferently in all cases, another technique which has the great advantage of disclosing them, no matter how few. This technique makes use of the principle of concentration (homogenisation) (*see Chapter 1*).

The specimen of sputum (5 to 20 cc.) should be as fresh as possible. The whole of it is mixed in a centrifuge tube with an equal volume of 50 per cent antiformin in sterile distilled water or, in the absence of the latter, with an equal volume of pure Javel water. After shaking for an instant there are added, for each 10 cc. of the mixture, 1.5 cc. of alcohol containing 10 per cent of chloroform. The tube is corked and again shaken briskly for some minutes, after which it is centrifuged. The supernatant fluid is poured off and the whole of the sediment carefully collected with a pipette or spatula and placed in the centre of a glass slide in the form of a large drop to which is added another drop of thymolated albumin and the mixture spread as regularly as possible. This is allowed to dry in the air or in the incubator and then fixed with heat and stained as described above.

The antiformin should be diluted with distilled water in order not to introduce any foreign acid-fast bacteria.

It should be recalled that some varieties of acid-fast bacteria, although quite rare in sputum, may be found in certain subjects (especially when milk and butter form a part of the diet) and, although after being stained by the method of *Ziehl* they appear less

distinctly red than true tubercle bacilli and ordinarily are more short and plump with ovoid thicker ends, they may nevertheless in some cases, give rise to regrettable confusion.

For this reason it is always to be recommended,—especially when the diagnosis is to be made in the face of doubtful clinical signs,—that the microscopical examination of the sputum be controlled by experimental inoculation.

3. Morphological characters of the bacilli

The morphology of bacilli in sputum is very variable. Most often they occur in the form of rods from 1 to 2 microns long, slender and uniformly stained; but now and then they may be short and plump, or again even two or three times as long as usual, extremely slender, granular, curved or straight. In certain cases they appear broken like a short chain of slender streptococci. Their number varies in different portions of the same sputum. They may be found isolated or in irregular clumps at random and one upon another like jack-straws or in small masses and bound together like a bundle of sticks. These various types may be encountered in the same patient if the sputum be examined at various intervals. They seem to correspond to special stages in the growth of the organism in the tuberculous lesions; smallness of the bacilli and capacity for homogeneous staining being an indication of an abundant growth, and of a young and vigorous development; the elongated form, slender and granular, indicating on the contrary a diminution in their number, but nothing as to the evolution of the focus from which they come.

Carl Spengler (of Davos) attributes to the long slender forms,—of which he has made a special type, the *Bacillus tuberculosis humanolongus,*—a greater virulence for both man and rabbit.

It would seem then that the study of the form of the expectorated bacilli is of very real interest. Some workers have begun it (Piéry and Mandoul,[3] Orth, Chauvin, Bezançon and P. Weil); but up to now it has barely been outlined.

Furthermore we know very little as to the prognostic value of the counting of bacilli, which vary greatly in number from day to day and from one hour to another in the same patient. It often happens, for example, that the bacilli are completely lacking during several

[3] Compt. rend. Soc. de biol., 1904, **57**, 586; 625; 1905, **58**, 99.

successive days and that all at once they are very numerous owing to the fact that one tubercle or several are simultaneously pouring their contents into a bronchus. Even in cavity cases bacilli are seen at times to disappear almost completely, while subjects with latent, very resistant forms, may regularly and constantly throw off organisms in abundance.

It may be said, however, that in general the progressive disappearance of the bacilli is of very favorable prognosis, whereas the elimination of large numbers of bacilli in clumps should arouse suspicion of a rapidly progressive evolution.

Several scales have been proposed with the object of comparing the number of bacilli in successive slides made from sputa of the same patient. At *Davos* there is generally employed that of Denoeyer which includes 10 numbers:

Number 1 corresponds to 1 to 4 bacilli in the whole of one preparation;

Number 2 corresponds to 1 bacillus in each microscopic field;

Number 3 corresponds to 2 bacilli in each field; and so on up to

Number 10 which corresponds to 9 bacilli or more in each microscopic field.

Löwenstein[4] thought that he found a relation between the arrangement of the bacilli within the leucocytes and the resistance of the individual. This phagocytosis, which indeed is observed but rarely in sputum, is according to him, of favorable prognosis. It should appear especially in subjects under treatment with tuberculin.

4. Mixed infections of tuberculous sputa

Expectorated material, even when collected with the greatest precaution as to cleanliness and examined quickly after being coughed up, shows almost always an abundance of other bacteria along with the tubercle bacillus. These microörganisms come from the upper air passages, the pharynx, and the mouth, often also from pulmonary lesions where they grow in the albuminous or purulent exudates after having been introduced either by the inspired air or the circulating blood.

P. Halbron[5] has made a valuable study of these mixed infection organisms and considers that direct examination after staining gives

[4] Deutsch. med. Wchnschr., 1907, **33**, 1778.
[5] Thèse, Paris, 1906.

better information than cultures as to their number, kind and proportion to tubercle bacilli. Thus one can estimate the relative frequency of the *pneumococcus*, of the *Micrococcus catarrhalis* and of the *enterococcus*, the bacteria most commonly found. At the same time one can in a measure judge the intensity of the secondary infection and its relation to the symptoms presented by the patient (fever, cough, abundance and nature of expectoration, blood infection, gastro-intestinal disturbances, intoxication, etc.).

In severely infected tuberculous cases the *Micrococcus tetragenus* is often found in abundance in the sputum; at times there are also found *streptococci*, *staphylococci*, a *pseudo-meningococcus*, the *pneumobacillus of Friedländer*, *B. proteus*, *Spirillum crassum*, *Vibrio tenuis*, etc., even *pseudo-diphtheria* bacilli or anaerobes. The latter, according to Veillon and Repaci,[6] may stamp the tuberculous infection with special characters, such as fetid sputum or gangrenous processes in the walls of the cavities; or they may even cause serious complications like pulmonary gangrene, putrid pleurisy and aggravation of the general condition. S. C. Harvey[7] finds that the *non-hemolytic streptococcus* is the most frequent pyogenic agent in pulmonary tuberculosis, at any rate in the United States.

In spite of the studies of Babès, Ramond and Ravaut, of Michelazzi, Prudden, Sata and those more recently made in my own laboratory by E. Duhot,[8] we are still poorly informed as to the rôle played by the majority of these bacteria in tuberculous lesions and upon the organism which harbors them. We are still ignorant as to their prognostic importance. It does seem certain, however, that they are not without harmful effects and that several varieties, particularly the strict anaerobes, are capable by their presence and through their diastasic secretions of aggravating the lesions produced by the tubercle bacillus. Therein lies a whole study which up to now has been scarcely touched (H. Kogel),[9] and the question presents itself whether there would not be an advantage in vaccinating the diseased body against the microörganisms whose multiplication may be prejudicial, by making use of the newer methods of preparation of autovaccines. This has already been attempted in some cases by Wolff-Eisner,

[6] Ann. de l'Inst. Pasteur, 1912, **26**, 300.
[7] J. Med. Research, 1917, **35**, 279.
[8] Compt. rend. Soc. de biol., 1914, **76**, 797.
[9] Internat. Centralbl. f. Tub.-Forsch., 1913, **7**, 369.

Hudson, Alexander, Passini and Wittgenstein, Hoffmann and Martin. Probably certain tuberculous cases would be better able to defend themselves against their bacilli were they not compelled to contend at the same time against other infections or other concomitant intoxications.

5. Control by experimental inoculation

If a susceptible animal develops a tuberculous infection following inoculation with a suspected sputum, we have proof of the existence of virulent bacilli in that sputum, even though microscopic examination has been negative. The indication therefore is to have recourse to this experimental inoculation in doubtful cases or where it might be useful in determining the human or bovine nature of the infecting virus.

The animal of choice is the guinea pig.

Inoculation should be made subcutaneously into one of the thighs in the neighborhood of the groin. With syringe or pipette, one should inject a bit of the muco-pus taken from the center of a freshly collected specimen of sputum. The suspension of this material should be prepared with a little sterile physiological salt solution.

Injection into the peritoneum must be carefully avoided since pyogenic bacteria or those from the saliva may lead to a fatal peritonitis.

If the sputum contains an appreciable number of bacilli (at least 40 to 50 in the quantity injected) they will fairly quickly set up a characteristic swelling of the inguinal gland nearest the point of inoculation. This gland, normally small and imperceptible through the skin, becomes hard, swollen and adherent to the surrounding tissue and, even in 10 to 15 days, may attain the size of a large pea. But this engorgement of the gland does not in itself suffice to establish the diagnosis of tuberculosis, since it may in certain cases,—although indeed quite rarely,—be induced by various pyogenic organisms existing in the sputum. Its specificity is actually demonstrated only by the extension of the infection to the visceral organs. It is therefore not enough to simply excise the gland and search for bacilli in sections; one should await the death of the animal, which occurs as a rule in 8 to 10 weeks, or else kill it when emaciation is unmistakable. At autopsy, there are then found macroscopically visible tubercles in the spleen, the liver, the lungs and in the different

gland groups. When sectioned the lymph node of the originally infected groin shows a caseous centre. A slide made from its contents, or from a fragment of spleen tubercle crushed and stained with *Ziehl*, removes all doubt if bacilli are disclosed.

The sputum used for inoculation may contain only a few bacilli, too few to produce an intense lymphatic reaction and to rapidly tuberculize the animal. In such a case the inguinal adenitis is wanting, emaciation does not take place and the guinea pig remains for weeks, sometimes even for months, in an apparently healthy condition. But if a sufficiently long period of observation, 4 months for example, be allowed to pass before killing the animal, autopsy then always shows some tubercles, either small or very large, localized in the spleen and in the liver, or in at least one of these organs.

This guinea pig method of control is the most certain. The only objection to it is that it does not give definite information until after a long delay. Naturally, many attempts have been made to reduce this period.

To this end Nattan-Larrier[10] proposed the inoculation of a few drops of a sputum suspension into the mammary gland of a lactating guinea pig. An actual culture *in loco* is then very rapidly brought about and, by the fifth or at least by the tenth day, if the gland is forcibly expressed, there may be squeezed from the milk ducts a drop of fluid which, if collected and examined microscopically after staining with *Ziehl* frequently shows many bacilli. The diagnosis can thus be established in about one week, but it is necessary to take certain special precautions to avoid infection of the gland by the various pyogenic bacteria associated with the tubercle bacillus. Nattan-Larrier recommends heating the sputum on two successive days for one hour at 54°C. But this procedure is open to question, since heating, even though not prolonged, modifies the virulence of the bacilli. Then too, the difficulty of always having lactating animals at hand takes away all practical value from this procedure.

If one is hurried, it is infinitely more simple and advisable to excise the inguinal gland after 10 to 14 days, to cut it into fine pieces with small scissors and grind in a sterile agate mortar. During the grinding the paste is dissolved by the addition of a small quantity (drop by drop with a pipette) of a 50 per cent antiformin solution in distilled water. The mixture is then centrifugated and the whole sediment examined for bacilli as described under sputum.

[10] Compt. rend. Soc. de biol. 1900, **52**, 1024.

6. Determination of human or bovine origin of bacilli contained in sputum

When the *probable source* of a tuberculous infection, whether *human or bovine*, is to be determined, one proceeds, as already described in Chapter XXI-B, preferably by the method of inoculation into the rabbit. Two or three cubic centimeters of a suspension of the sputum may be injected directly under the skin of the belly or behind the shoulder.

The rabbit is but slightly susceptible to the human virus. When inoculated with organisms of this type, the animal presents a moderate swelling of the glands draining the inoculated region, but remains healthy and does not emaciate. If killed two months later, there are found at the point of inoculation a few caseous masses in which bacilli are contained; now and then there are a few tubercles in the lungs and in the spleen or liver;—small lesions with but little tendency to spread and with which the rabbit would have been able to survive for a long time.

On the other hand, *if the virus is of bovine type,* the *lesions as a rule spread rapidly;* the animal emaciates and dies of generalized tuberculosis within 8 to 12 weeks.

It is almost universally admitted today that the sputum of phthisical patients contains only bacilli of the *human* type. Only exceptionally are bacilli encountered which present the cultural and virulence characters of the *bovine* type. In 1912, at the KK. *Gesundheitsamt* at Berlin, E. A. Lindemann[11] assembled all the cases reported up to that time, including those of Park and Krumwiede of the United States and those of the *English Royal Commission;* there were 790 determinations in all, among which the human bacillus was found 784 times and the bovine bacillus in only 3 instances (one being doubtful, that of De Jongh-Sturmann); of the remaining three cases two were mixed and one was questionable (that of Mohler and Washburn) (*see Chapter XXV*).

From this finding it should not be concluded that pulmonary tuberculosis in man is due exclusively to infection by the human bacillus. The only fact which one is justified in asserting positively is that the bacilli isolated from sputa, and derived from more or less long-standing pulmonary lesions, show the cultural and virulence

[11] Tuberk.-Arb. a. d. k. Gsndhtsamte, 1912, H. 12, 11.

characters usually presented by the human type. But it is possible that the bacilli of bovine origin, having sojourned for many years in the body of the human subject and having multiplied through many successive generations until pulmonary lesions were at length produced, have eventually acquired the same characters of culture and of virulence as those of the human type, with the result that the methods which we use for differentiation furnish results whose interpretation becomes impossible (*see Chapter XXI*).

B. EXCRETION BY THE INTESTINE AND BILE DUCTS

The fact is today well established that many bacteria in circulation in the blood may be eliminated through the intestine.

Emmerich,[12] and then Buchner[13] had shown, in 1885, that the vibrio of cholera, if injected into the blood or subcutaneously, can be recovered a few hours later in the intestines. Issaeff and Kolle,[14] 12 years later, confirmed these results by inducing by the same procedure a diarrhea with multiplication of the cholera vibrios in the fecal matter; but none of these authors had associated this fact with the bile duct, thus leaving their experiment without value as a demonstration, since the presence of bacteria in the intestine may be attributed to the excretory rôle of the liver. It was necessary to exclude the intervention of this organ and this was done by Ribadeau-Dumas and Harvier[15] by tying off the common bile duct.

Hess[16] even took the precaution of also ligating the canal of *Wirsung* in order to prevent elimination by way of the pancreas. In three rabbits the duodenum was divided between two ligatures immediately below the common bile duct, and the canal of *Wirsung* tied off. By this procedure he avoided at one and the same time a biliary stasis and any communication of the intestine with the respiratory passages. *Bacillus prodigiosus* was injected intravenously and recovered in the small intestine of two of the animals thus operated upon. Finally, in order to do away with any possible influence of operative shock, the same investigator created in a dog a duodeno-cutaneous fistula with two duodenal openings, one of them above the

[12] Arch. f. Hyg., 1885, **3**, 291.
[13] Ibid., 1885, **3**, 361.
[14] Ztschr. f. Hyg., 1897, **18**, 17.
[15] Compt. rend. Soc. de biol., 1910, **69**, 181.
[16] Arch. Intern. Med., 1910, **6**, 522.

point of ligation, the other below. Through the upper tract gastric contents, bile and pancreatic juice were expelled; through the lower the animal was fed during two days. Hess now closed both openings and injected *Bacillus prodigiosus* into the jugular vein. Two hours later the dog was killed and cultures revealed the microörganism in the upper and lower parts of the ilium, never in the caecum and large intestine.

By means of another technique I proved, collaborating with C. Guérin,[17] that the tubercle bacillus can likewise be eliminated by the bile ducts. We injected into the marginal ear vein of a series of rabbits, one centigram of finely suspended bovine bacilli from a six weeks old culture upon glycerin potato. The animals were killed *by cutting the throat*, 24 hours, 48 hours, 3, 4, 5, 6 and 7 days respectively after inoculation. The body was immediately opened and, with every care taken not to touch the surface of the liver, the contents of the gall-bladder were aspirated with a pipette and centrifugated. The sediment from the centrifugation was diluted with 2 cubic centimeters of physiological salt solution and inoculated in a dose of 0.5 cc. subcutaneously into the thighs of 4 guinea pigs. The same procedure and tests were carried through for each rabbit. All of the guinea pigs which received bile from the rabbits sacrificed 24 and 48 hours, and 5 and 6 days after injection, remained free from infection, while of the 4 which received the bile from the rabbit of the third day, 2 were tuberculous as were 1 of the 4 which received the bile of the rabbit of the fourth day, and 3 of those which received the bile of the rabbits killed on the seventh day.

It is evident therefore that a part of the bacilli introduced into the blood stream may be eliminated through the liver and evacuated with the bile through the intestine.

We performed another experiment which was still more conclusive.[18] In two heifers we made a permanent biliary fistula which enabled us to draw off from the gall-bladder at will, with the aid of a pipette, the quantity of bile necessary for test inoculations (*fig. 28*).

One of the heifers received 3 milligrams of virulent bovine tubercle bacilli into the jugular vein. Each day, before and after the experiment, a small quantity of bile was withdrawn from the gall-

[17] Compt. rend. Acad. des sci., 1909, **148,** 601.
[18] Ann. de l'Inst. Pasteur, 1913, **27,** 162.

bladder and 0.5 cc. injected into each of 4 guinea pigs. Of the total of 109 animals, 15 became tuberculous, all of which had received bile collected more than 19 days after the inoculation of the heifer. This animal died of acute miliary tuberculosis on the twenty-eighth day.

When one considers the minute quantity of bile injected subcutaneously into each guinea pig, 0.5 cc. (as much as a guinea pig will tolerate), and if one compares it with the enormous volume (about 2 litres) of this fluid excreted by the heifer in twenty-four hours, one is

Fig. 28. Permanent Biliary Fistula in a Heifer

A large quantity of bile may be daily collected from the gall-bladder, with a pipette, and examined for tubercle bacilli being eliminated into the intestine.

forced to conclude that the number of bacilli evacuated through the bile passages of this animal is certainly considerable!

In parallel manner we tested the virulence of the dejections of another heifer which had received human bacilli intravenously. Of 66 guinea pigs, each inoculated with 0.1 gm. of fecal material, only 3 became tuberculous; but it should be here observed again that the quantity of excrement received by each animal was infinitely small in comparison with the amount evacuated by the heifer in the course of 24 hours (about 7 to 8 kgms.).

E. Joest and E. Emshoff[19] have proved likewise the frequency of elimination of bacilli by the bile of animals *naturally infected.* From this point of view they studied, by means of guinea pig inoculations, the bile of tuberculous cattle and swine.

The results obtained were the following: in 14 of 57 cases (24.4 per cent), the bile infected the guinea pigs. Of these 14 positive cases, the bacilli could be demonstrated 4 times by microscopic examination. Almost always there was a generalized tuberculosis in the cattle or swine with hepatic lesions. Only twice were the latter lacking; there existed however, to make up for them, tuberculous lesions of the periportal glands.

One may therefore conclude, according to Joest and Emshoff, that, in cattle and swine affected with generalized tuberculosis, there is an elimination of virulent bacilli by way of the bile passages in 25 per cent of the cases. It is probable that the percentage is still higher and that, in a certain number of experiments, the results were negative because of the small quantity of bile injected in order to avoid causing serious necrosis in the guinea pigs.

Other experiments confirming the same facts have been carried out with guinea pigs by M. Breton, Mezie and Bruyant in my laboratory.[20]

Attention had been called a long time before to the presence of bacilli in the dejections of phthisical patients and in those of tuberculous animals, but there was a tendency to agree with Cadéac and Bournay,[21] Wood, Lichtheim, Shaw, and Anglade that this was the result of swallowing the sputum, the ingestion of virulent material, or of the existence of intestinal lesions. The studies of Fraenkel and Krause, of Emerson and particularly those of Rosenberger[22] (1907–1909) suddenly enlarged the problem. These authors found acid-fast bacilli in a large number of subjects affected with miliary or with closed tuberculosis.

There was a doubt as to whether the bacilli were true tubercle bacilli, and D. Moore Alexander,[23] Philip and Porter,[24] Rittel-

[19] Ztschr. f. Infektionskr. . . d. Haustiere, 1912, **12**, 117.
[20] Compt. rend. Soc. de biol., 1912, **73**, 58; 118.
[21] Ibid., 1895, **47**, 795.
[22] Am. J. Med. Sci., 1907, **134**, 830; 1909, **137**, 267.
[23] J. Hyg., 1910, **10**, 37.
[24] Brit. M. J., 1910, ii, 184.

Wilenko,[25] A. T. Laird, G-L. Kite and D. A. Stewart[26] undertook methodical studies of the question as it affects man.

For each specimen of feces which he examined, Alexander weighed out 1 gm. in a watch glass, ground it in a mortar, diluted with a few cubic centimeters of physiological salt solution and injected 1 and 2 cc. quantities into guinea pigs (that is to say, 0.01 and 0.02 gm.). Of 24 specimens from cases of pulmonary tuberculosis, 23 showed themselves virulent.

In 2 fecal specimens from subjects suffering from lupus and in 129 from non-tuberculous cases, acid-fast bacilli were not once found by microscopic examination. The author concluded from this that whenever acid-fasts are found in human dejections, they are true human tubercle bacilli. He found the latter 52 times in 74 individuals affected with various non-open tuberculoses (pleurisy, miliary tuberculosis, caseated glandular forms, meningitis, bone and joint tuberculoses). In the same persons the bacilli were encountered only intermittently and their evacuation was often provoked by the taking of calomel or other cholagogues.

Philip and Porter sought the bacilli in the fecal matter of 100 cases of pulmonary tuberculosis. Acid-fasts were found in 75. There were 42 of these tuberculous cases who were not expectorating any bacilli, but 29 of them showed bacilli in their stools. On the other hand, all of those who were expectorating bacilli had them also in the feces.

Laird, Kite and Stewart found 48 specimens virulent among 87 in which direct examination by the antiformin method had revealed the presence of acid-fasts.

The technique most to be recommended in searching for tubercle bacilli in feces is the following, which I have myself employed with C. Guérin and which H. Thieringer[27] also used:

Thirty grams of material are weighed out in a conical vessel and 55 cc. of sterile water and 15 cc. of antiformin mixed with it. The mixture is shaken several times and allowed to stand for 3 to 4 hours, after which it is centrifugated and the supernatant fluid poured off. The sediment is collected in a sterile vessel and diluted with 8 to 10 cc. of physiological salt solution. It is next filtered through 2 or 3

[25] Wien. klin. Wchnschr., 1911, **24**, 527.

[26] J. Med. Research, 1913, **29**, 31.

[27] Arb. a. d. k. Gsndhtsamte, 1912, **43**, 545

double layers of sterile gauze and inoculated in doses of 2 to 3 cc. into 3 or 4 guinea pigs, under the skin in the inguinal region.

Several recent reports have brought out the importance of tuberculous contamination of cattle through fecal matter and also the rôle of the bile ducts in the excretion of bacilli.

E. C. Schroeder and W. F. Cotton,[28] of the *Bureau of Animal Industry* at Washington, published the results of very numerous and suggestive experiments indicating that the best method for surely bringing about infection in swine consists in feeding them, with their food, fecal matter from tuberculous cattle. They also proved that 40 per cent of cows which react to tuberculin but which exhibit no clinically demonstrable lesion, eject virulent bacilli intermittently in their dung.

Elmer C. Peterson,[29] and Reynolds and Beebe[30] find, in their experience, that cattle which react to tuberculin but which exhibit no clinically demonstrable lesions, do not expel bacilli in their dejections. On the contrary, A. T. Peters and C. Emerson,[31] working with 22 animals not clinically tuberculous, but which gave a positive tuberculin reaction, discovered bacilli in the dung 3 times (i.e., in 7.31 per cent) by inoculating 0.5 cc. into the guinea pig.

Joest and Emshoff,[32] in Germany, found bacilli in the bile of 26 cattle and of 31 swine. At the Berlin abattoir, C. Titze and E. Jahn had the same result. In their investigations, the bile of 42.3 per cent of tuberculous cattle and goats showed itself virulent for the guinea pig, even though the lesions were not at all extensive macroscopically and at times were limited to different groups of glands.

The same authors, collaborating with H. Thieringer,[33] noted that animals with open tuberculous lesions eliminate many bacilli: but they were not able to find them in the dung of cattle reacting to tuberculin but without clinically demonstrable lesions. They used 30 gms. of fecal material with 15 cc. of pure antiformin and 55 cc. of sterile physiological salt solution. After being left to stand for 2 hours

[28] U. S. Dept. Agric., Bur. Anim. Indust., Rep., 1906/07.

[29] Rep. New York State Veter. Coll., 1909/10, p. 65.

[30] Bull. No. 103, Minn. Exper. Station.

[31] 22nd. Rep. Agric. Exper. Station of Nebr., 1909.

[32] Arb. a. d. k. Gsndhtsamte, 1913, **44**, 35.

[33] Ibid., 1913, **43**, 1.

the mixture was centrifugated 3 times before the sediment was inoculated. By thus examining 28 animals with pulmonary or only glandular tuberculosis, they found the bacilli in 11 cases; while of 68 animals which had served for the most part for various vaccination experiments and which all reacted to tuberculin, though clinically free from tuberculosis, bacilli could never be demonstrated in the dejections. They do not indicate whether the test was repeated several times in the same animal.

In conclusion, Lydia Rabinowitsch[34] reports that in 17 tuberculous cattle in different stages she was able to isolate tubercle bacilli 12 times from the gall-bladder. In 8 of the cases the animals had intestinal lesions and in a single one there were lesions of the liver.

One must therefore conclude that the bacilli are frequently transported to the liver in the circulating blood and eliminated through the bile. The presence of virulent bacilli, often in great numbers, in the fecal matter, whether they have this origin or are derived from swallowed sputum, is assuredly an important source of danger and plays a capital rôle in the dissemination of tuberculosis. It explains how contamination is effected in stables; it shows how infection of cow's milk may be brought about,—even that of healthy cows,—by particles of fecal matter in the course of milking and, as regards the human race, it calls our attention to the possible danger of foods (in particular of vegetables cultivated in fields and gardens where manure is used), of linen, of clothes and of objects of all sorts soiled by the excrement of those who are tuberculous or are carriers of bacilli, although apparently healthy.

Undoubtedly it is in large part in this way that tuberculosis propagates itself on farms, in rural communities and in the crowded and often unhygienic groups (asylums for the insane, prisons, etc.), and also among native populations of foreign lands into which the virus is borne by traders and travellers coming from regions long since contaminated.

C. EXCRETION IN THE URINE

The renal filter in a state of health is impermeable for bacteria. But the bacillemia, which we recognize today as so frequent at all stages of tuberculosis, often sets up minute nonfollicular renal lesions

[34] Deutsch. med. Wchnschr., 1913, 39, 103.

through which the tubercle bacilli may find their way even into the urine. And there is all the more reason for this passage to take place when the glomerular capillaries become the seat of caseated tubercles. It is then accompanied by a more or less abundant elimination of cellular elements and principally of leucocytes.

The presence of bacilli in the urine may therefore be demonstrated under many circumstances, even when renal tuberculosis properly speaking does not exist. Many clinicians, among whom I would cite Durand-Fardel, Landouzy, Tilden-Brown, and Wechselbaum, brought out this fact long ago, and the more recent bacterioscopic researches of Foullerton and Hillier,[35] Fournier and Beaufumé,[36] of A. Jousset,[37] of Supino,[38] of Bezançon and Philibert[39] would have furnished abundant confirmation had they not all been subject to the same criticism; namely, that they were based only upon direct examination of slides of stained centrifugated sediments. This method in fact does not permit even the most experienced observer to entirely avoid errors since certain organisms of the acid-fast group are not uncommonly encountered in urine. The most frequent of these bacilli is that of the preputial smegma, announced in 1884 by Lustgarten and carefully studied by Alvarez and Tavel, Doutrelepont and Schultze, Matterstock, Grunbaum, and others. According to the last author it is found in 59 per cent of normal urines in women, somewhat less frequently in men. Of this organism there are two varieties, one slender and delicate, much like the tubercle bacillus, the other granular and ordinarily to be found in clumps or in rods lying parallel like diphtheria bacilli.

According to Bezançon and Philibert they can be easily differentiated from the true tubercle bacillus if care is taken to allow the one-third strength nitric acid to act upon the *Ziehl* stained slides for two minutes and the absolute alcohol for 5 minutes. The smegma bacilli should then be decolorized while the tubercle bacilli retain the dark red of the fuchsin.

This method—and the same can be said for that of Dahms which I described in Chapter I (E)—is not reliable, and it is better in all

[35] Brit. M. J., 1901, ii, 774.
[36] Compt. rend. Soc. de biol., 1902, **54**, 1258.
[37] Arch. de méd. expér., 1904, **16**, 521;—Semaine méd., 1904, **24**, 293.
[38] Riforma méd., 1905, **21**, 561.
[39] Bull. Soc. d'étude scient. sur la tuberc., 1908, February.

cases to resort to experimental inoculation, despite the uncertainty which may arise from the bacilli being too few to quickly tuberculize the animals.

One should, in so far as possible, collect the urine separately from each ureter and centrifuge it immediately in large sterilized tubes, employing for the test a total volume of about 100 cc. The sediments are to be suspended in a little physiological salt solution and injected into at least two guinea pigs, under the skin of the thigh or near the fold of the groin, as in the case of sputum and with the same technique.

If the characteristic adenopathy appears by the twelfth or fifteenth day, one may either excise the gland to examine for tubercle bacilli in the sections, or wait until the infection becomes generalized before autopsying the animals.

Frequently the inoculation test is positive when microscopic examination of the centrifugated sediment has been negative. Pagès,[40] for example, in 91 cases of renal tuberculosis confirmed later by operation, found bacilli only 22 times on direct examination, whereas inoculation was positive in 82 patients.

Bertier[41] studied the urines of 24 subjects, all severely affected with pulmonary tuberculosis, the majority febrile but not suspected of having renal lesions. Some had a little albuminuria. He obtained 8 positive results by the inoculation test, that is to say 33 per cent. All of the guinea pigs which became infected had received the centrifuged sediment from the urine of cavity cases which were progressive but of which only one had albumin. There does not seem therefore to be a direct relationship between albuminuria and bacilluria.

In the urine of children suffering from different diseases (nephritis, pleurisy, broncho-pneumonia, meningitis, miliary tuberculosis, etc.), P. Nobécourt[42] discovered bacilli by inoculation in only 4 patients among 37. Positive results were secured with two children who had renal tuberculosis, a third was febrile with tuberculosis of the lungs and cavity formation, and the fourth had a miliary tuberculosis with meningeal reaction. Of 8 guinea pigs, 5 were tuberculized with quantities of urine varying from 60 to 150 cc. The urines of patients

[40] Thèse, Lyon, 1906.

[41] Bull. Soc. d'étude scient. sur la tuberc., 1909, December.

[42] Assoc. française de pediat., 1911, October.

suffering from nephritis of various types, with or without hematuria, gave constantly negative results.

Among adult cases of pulmonary tuberculosis, bacilluria is also rare. Léon Bernard found it only 5 times in 42 subjects, the positive findings bearing no relation to the presence of albuminuria.

This rarity is in contrast to the frequency of bacilluria observed commonly in the various forms of tuberculosis of childhood. When bacilluria exists it does not justify one in asserting that a renal lesion is present unless the bacilli are very abundant and accompanied by pyuria. Direct microscopic examination is then of great value.

D. EXCRETION BY THE MAMMARY GLANDS

Like the *normal* kidney, the *normal* mammary gland does not permit the passage of bacteria. But blood infections and particularly a tuberculous bacillemia, may lead to the formation of small inflammatory foci round about one or several functionally active glandular *acini*, or bring about tuberculous lesions. In both cases the leucocytes which are capable of transporting the bacilli pass into the lacteal secretion.

Many investigations, upon which we have dwelt in connection with bovine tuberculosis and its transmission to man (*Chapter XXV*), have made evident the dangers of mammary gland tuberculosis from the standpoint of milk contamination. We are less accurately informed as to the frequency of filtration of bacilli through the gland, when the latter itself, or the lymph nodes which drain it, are free from demonstrable lesions on clinical examination or autopsy.

The question can be settled only by guinea pig inoculation, by a large number of experiments and by protracted observation of the same individuals, both the mothers and the infants. At the present time we lack in large measure all of these facts.

Certain investigators, among whom should be cited particularly Ostertag, Fiorentini and Ceradini, Ascher, Leclainche and Morel, regard milk from tuberculous cows with healthy udders as devoid of virulence. On the other hand Lydia Rabinowitsch and Kempner, Karlinski,[43] in Germany, Moussu in France, John Mohler, Schroeder and Cotton in the United States, and Sheridan Delépine of the Royal English Commission have published facts which prove indisputably

[43] Ztschr. f. Thiermed., 1905, 9, 414.

that *cows, and also goats, apparently free from any mammary lesion, but reacting to tuberculin, expel bacilli now and then in the milk which they secrete.*

Histological studies of Joest and Kracht-Palejeff[44] had already demonstrated that in 25 per cent of cows affected with generalized tuberculosis, but without apparent mammary gland involvement, one finds definitely tuberculous microscopic lesions in these glands. More recently, Ishiwara,[45] by experimental inoculation, found bacilli present in 5 of 26 perfectly healthy mammary glands which he examined, and in which microscopical examination had given negative results.

In a series of 57 inoculations into guinea pigs, Moussu[46] obtained 7 positive results. Rabinowitsch, and Schroeder and Cotton demonstrated that calves, born of apparently healthy but tuberculin positive cows, and suckled by the mother, react to tuberculin after 2 to 6 months. It appears then that, as regards the bovine species, no doubt can remain and that, as Moussu would have it, the milk from every milch cow giving a positive tuberculin reaction must be regarded as suspicious.

Experimentally, Titze, at the KK. *Gesundheitsamt* at Berlin, has shown that when tubercle bacilli are injected intravenously into a lactating cow, the bacilli begin to appear in the milk at about the third week, and they persisted, in one animal, up to 144 days. In another experiment the excretion began after 24 hours and the bacilli could not be recovered afterward until the ninety-ninth day. In these two cows the posterior left udder alone was found infected.

In the human race the results do not appear to be different. Forster, Mathilde de Biehler,[47] Schlossmann, and Auché,[48] were unable to tuberculize guinea pigs by inoculating them either subcutaneously or intraperitoneally with 5 to 15 cc. of milk from women suffering from severe pulmonary tuberculosis. On the contrary, Escherich, Roger and Garnier, Guillemet, Rappin, Fortineau and Patron,[49]

[44] Ztschr. f. Infektionskr . . . d. Haustiere, 1912, **12**, 299.
[45] Centralbl. f. Bakt., 1913, **70**, 1.
[46] Compt. rend. Soc. de biol., 1904, **56**, 617; 1905, **58**, 310.
[47] Arch. de méd. des enfants, 1908, **11**, 473.
[48] Compt. rend. Soc. de biol., 1913, **75**, 594.
[49] Ibid., 1906, **61**, 25;—Thèse, Paris, 1909.

O. Füster (of Vienna),[50] and Moussu[51] under analogous conditions or with larger quantities of centrifugated milk proved that this milk now and then did contain the bacilli. Rappin found it infectious twice in four times, even in an amount of 2 cc.; Moussu using 25 to 50 cc., only once in 10 times. But Kurashige, Mayeyama and Yamada,[52] in a series of experiments carried out with 20 tuberculous women, found the bacilli in 17 cases (85 per cent). Of these 20 women, 9 were in an early stage of the disease or even in the so-called pretuberculous stage. All of the 9 had the bacillus in the blood and 6 of them had it in the milk. Of 5 tuberculous women in the second and third stages (according to Turban), bacilli could be disclosed simultaneously in the blood and in the milk (100 per cent).

The Japanese authors remark at the same time that, in every one of these nursing women, the number of bacilli contained in the milk was very low (2 to 7 per 5 cc.) and that the general condition of their infants was satisfactory in the majority of cases. The danger for the nursing children therefore did not seem extremely grave. It may be asked whether the fact that the infants were thus absorbing but a few bacilli in small number did not confer upon them a certain degree of resistance to the serious infections to which they would be exposed later on. If this were the case it would be very advantageous for the infant of a mother with non-open tuberculosis (therefore incapable of communicating a massive infection through the sputum) to be fed by her, at least during the first weeks following birth.

Be that as it may, it seems established that although excretion of bacilli by the mammary glands of tuberculous females is rather rare it may nevertheless occur in certain cases, even in the absence of a local lesion.

[50] Wien. klin. Wchnschr., 1906, **19**, 588.
[51] Compt. rend. Soc. de biol., 1906, **61**, 171.
[52] Ztschr. f. Tuberk., 1912, **21**, 433.

CHAPTER XXXIV

ACCESSORY REACTIONS FOR THE DIAGNOSIS OF TUBERCULOUS INFECTION

A. ALBUMIN REACTION

Many years ago it was shown (Caventou,[1] Biermer, Benk) that the products of expectoration, in certain diseases, contain albumin in greater or lesser abundance. But Wanner[2] was the first to have the idea of making a special study of the subject in the different pulmonary infections.

According to him there is no albumin to be found, or it exists only in imperceptible traces in the sputum of essential asthma and in that of simple acute or chronic bronchitis. On the other hand, as much as 2 or 3 decigrams per 100 gms. of sputum are usually to be found in pneumonia and at least 0.01 to 0.1 gm. per 100 in the sputum of tuberculosis, even when the lesions are only slightly advanced.

Wanner employs an excellent technique: he recommends that the mucin and cellular débris be first removed by mixing the sputum with an equal volume of 3 per cent acetic acid solution. After shaking and filtering through paper he adds sodium hydroxide to the filtrate until the reaction is weakly alkaline, and then a little sodium chloride. He then coagulates with heat, collects the coagulum upon a filter, washes it with hot water, with alcohol and then with ether, and weighs it after desiccation in order to obtain the exact proportion of albumin in relation to the initial quantity of expectorated material.

In 1909 H. Roger,[3] alone or in collaboration with Lévy-Valensi and later with B. Mikhailoff, systematically examined the sputa of a large number of patients for albumin and drew the attention of clinicians to its almost constant presence in open pulmonary tubercu-

[1] Bull. Acad. méd., 1843, **8**, 779.

[2] Thèse, Bâle, 1903.

[3] Bull. et mém. Soc. méd. d. hôp. de Par., 1909, 3. s., **28**, 427;—Presse méd., 1910, i, 289.

losis. "One may," says this scientist, "divide expectorations into two groups. Those of the first group contain no albumin; they are due to a relatively abundant secretion of bronchial mucus and are associated with simple, acute or chronic bronchitis and with pulmonary emphysema. The others, which contain albumin, are evidence of a more severe process; they are to be associated with an inflammation or with an exudation; and they enable one to eliminate a simple bronchitis."

For the quantitative analysis of albumin, H. Roger advises that the expectoration be collected in clean sputum receptacles on awaking in the morning, in order that it may be as free as possible from saliva or food débris. The sputum is mixed with an equal volume of water to which has been added a few drops of acetic acid to coagulate the mucin and nucleo-albuminoids. It is then carefully triturated with a glass rod.

Too much acid should not be used, since such excess would prevent the albumin from precipitating later, but enough must be added to thoroughly dissolve the mucus. It is impossible to indicate exactly the necessary quantity, since the amount varies from case to case. In order to avoid error, it is well, after having filtered the fluid, to add another one or two drops of acetic acid. If the mucus has been entirely coagulated no clouding will be produced.

"Filtration is performed through ordinary filter paper or preferably through Chardin paper. If the masses have been carefully eliminated the filtration takes place very rapidly in one or two minutes."

To detect the albumin, Roger employs either heating (after the necessary addition of a little salt) or ferrocyanide of potassium in saturated aqueous solution which gives an extremely clear-cut reaction in the acetic medium: one drop is enough to give an abundant precipitate. One cubic centimeter of the reagent is placed in a test tube and the sputum filtered directly upon it. If the sputum contains albumin, a characteristic ring is seen to form quickly at the point of contact of the two liquids.

The ferrocyanide has the disadvantage of precipitating not only albumins but also the albumoses, so that as a rule nitric acid is today used in preference (Lesieur and Privey, Smolizanski), or the technique of Wanner (F. Bezançon and I. de Jongh) which has been described above is employed.

H. Roger and Mlle. Wourmann[4] proposed to determine separately the quantity of serum-albumin and globulin in the following way:

"The expectoration is carefully measured out in a graduated vessel. According to its consistency it is diluted with the same amount, or twice as much, of distilled water; a few drops of acetic acid are next added to coagulate the mucin and nucleo-albumins and the whole is filtered.

"The filtrate is divided into two parts: one is brought to boiling in order to coagulate all the albumins. It is poured upon a previously weighed filter paper and washed with boiling water acidulated with acetic acid. The filter is dried and weighed.

"The second part is carefully neutralized and magnesium sulphate added in the proportion of 80 gms. per 100 cc. of liquid. The volume is measured and, the globulin having been allowed to precipitate and collect for a certain time, the whole is poured upon a filter. A definite quantity of the filtrate is collected, slightly acidified with acetic acid and then boiled and poured upon a weighed filter paper. The precipitate is washed with acidulated distilled water until the liquid which passes no longer precipitates with a solution of barium chloride.

"It remains then but to dry the filter and weigh it. The weight of the serum albumin is thus obtained and the difference is the weight of the globulin."

One may also, like Smolizanski,[5] use the procedure of Hammarsten which consists in precipitating the globulin with a saturated solution of sulphate of magnesia.

The sputum filtrate is divided into two equal portions. To the first part are added two drops of phenolphthalein as indicator, after which it is neutralized with a decinormal sodium hydroxide solution, added drop by drop. Then after being filtered the mixture is saturated at room temperature with pure magnesium sulphate crystals (100 grams of magnesium sulphate to 100 cc. of liquid). It is shaken to favor solution of the salt and left to stand for 24 hours. The precipitated globulin is collected upon a filter which has been dried and weighed beforehand. This precipitate is next washed with a saturated solution of magnesium sulphate until the wash liquid no longer precipitates with heat or nitric acid. The filter is then left at 110°C. for a few hours, at which temperature the globulin coagu-

[4] Thèse, Paris, 1909.

[5] Thèse, Paris, 1911.

lates. The filter and its contents are next washed in boiling water which dissolves only the magnesium sulphate; the washing being continued until the filtrate no longer precipitates with barium chloride. The procedure is ended by washing in alcohol and ether. Finally the filter and its coagulum are dried in the incubator and weighed. The weight obtained minus that of the filter gives the proportion of globulin contained in one-half of the volume of sputum being tested. The filtrate saturated with magnesium sulphate is acidulated with acetic acid and brought to boiling. The serum albumin coagulates. It is collected upon a weighed filter, washed to remove the salt, dried in the incubator and weighed. From the weight thus determined, that of the filter is deducted and the amount of serum albumin in the portion of expectoration employed is thus obtained.

The method is simpler if one dispenses with purifying and weighing the globulin. It is enough to calculate the quantity of total albumin in a part of the filtrate and then to determine the quantity of serum albumin in the magnesium solutions from the filtering and washing of the other portion.

By subtracting, the weight of the globulin is obtained.

Diagnostic value of the albumin reaction

Albumin is found in the sputum in the course of acute infections of the lung, in pneumonia, in broncho-pneumonia, acute pulmonary congestion, passive congestions associated with cardiac disturbances, and in the edema of Bright's disease. But it is also found and almost constantly in all cases of active pulmonary tuberculosis, in almost every sputum which contains tubercle bacilli and at times even when no bacilli are expectorated.

On the contrary, it is not found in simple acute or chronic bronchitis, in emphysema, nor in miliary tuberculosis where the miliary tubercles contained in the lung parenchyma do not cause the formation of an exudate tending to be evacuated through the bronchi.

Numerous observations made first by H. Roger, later by Dieudonné[6] at Leysin, Geeraerd[7] at the dispensary Albert-Elisabeth at Brussells, by C. Ferreira[8] in Brazil, by Smolizanski, Guinard, Raymond

[6] Rev. méd. de la Suisse rom., 1910, April.

[7] Tuberculosis, 1910, **9**, 372.

[8] Presse méd., 1911, i, 309.

Letulle,[9] Courtois,[10] and others, attest that the albumin reaction is an excellent method for following the evolution of pulmonary tuberculosis. The albumin is said to disappear from the sputum when the foci become inactive and to reappear temporarily or constantly when an inflammatory process again intervenes. It reappears also as a transitory condition in patients who are reacting to a subcutaneous injection of tuberculin.

It seems that the quantity of albumin is almost always in proportion to the extent and gravity of the lesions. In patients with considerable softening there is often found one decigram and even more per 100 gms. of sputum.

The variety of albumin which predominates in the expectoration is of great importance in the prognosis (Mlle. Wourmann). Serum albumin is observed to prevail in rapidly progressive cases while globulin is found in excess in cases with a tendency to healing.

H. Roger[11] showed that an extract prepared from sputa of pulmonary tuberculous or pneumonic cases, when inoculated intravenously into a rabbit, causes a fall in the arterial pressure, while an extract of expectoration from cardiac cases is devoid of activity. From this it may be deduced that there is a "leucocytosis" of pulmonary origin, *hypotensive*, and another of blood origin, *non-hypotensive.*

But if the albumin contained in the sputum of pneumonic and tuberculous cases is coagulated with heat, the hypotensive effect upon the rabbit is lacking and the injection causes the arterial pressure to increase.

The value of the albumin reaction has been questioned or denied by several authors; Wanner, Prorok, F. Bezançon,[12] Remlinger,[13] E. Hempel-Jorgensen,[14] St. Acs-Nagy,[15] and A. Schneider. It would indeed be unjust to deny it any value whatever on the ground that it is lacking when the expectoration contains no leucocytes and that it is incapable of disclosing an occult tubercle bacillus infection. In

[9] Thèse, Paris, 1912.
[10] Thèse, Lille, 1913.
[11] Compt. rend. Soc. de biol., 1913, **75**, 103.
[12] Bull. Soc. d'étude scient. sur la tuberc., 1911, May
[13] Compt. rend. Soc. de biol., 1911, **70**, 358.
[14] Beitr. z. klin. d. Tuberk., 1913, **26**, 391.
[15] Wien. klin. Wchnschr., 1912, **25**, 1904.

my opinion it can be of service to the clinician by reason of the very great simplicity of its technique and because it may give prognostic indications of which one would regret to be deprived.

The principal fault is the fact that bronchial sputum is required, and very often this is lacking at the beginning of a pulmonary tuberculosis when the importance of diagnosis is the greatest.

B. ABDERHALDEN REACTION

This reaction, which Abderhalden first applied in 1912[16] to the diagnosis of pregnancy, has since been studied by a large number of investigators who saw in it the possibility of determining functional alterations of different organs.

Its principle is based upon the finding that a normal organ permits directly assimilable products resulting from a complete decomposition of the fats, carbohydrates and proteins to diffuse into the blood, whereas a *diseased* organ more or less inundates the body fluids, and particularly the serum, with incompletely disintegrated products which there represent actual foreign bodies. The latter not being assimilable, tend to be decomposed into assimilable products through the intervention of cellular ferments, for example by the ferments destructive of albuminoid matters, which then are poured in excess into the serum.

The reaction of Abderhalden has as its object the detection of these ferments; and it may be performed by two methods; the dialyzation technique and the optical method.

1. The dialyzation method

This method is based upon the fact that albumin does not pass through dialyzing membranes, while peptone, amino acids and their components, urea, etc., pass relatively easily through certain of them.

If pure albumin from any healthy organ whatever (liver, spleen, or kidney for example) and the serum of a healthy subject are placed in an appropriate dialyzer, neither the albumins from the healthy organ nor those of the healthy serum will pass through the membrane. But if the organ is diseased, its albumins are to a greater or lesser

[16] *Schutzfermente des tierischen Organismus; ein Beitrag zur Kenntnis der Abwehrmassregeln des tierischen Organismus gegen körper-, blut- und zellfremde Stoffe.* Berl., 1912, Springer.

degree decomposed by the ferments normally present in the serum, and the products of the decomposition (peptones, amino-acids, etc.), may be detected by certain chemical reagents in the dialysate.

This method requires the use of dialyzers completely impermeable to albumins and permeable to peptones. It necessitates also a particularly difficult and delicate preparation of the pulp of the organ to be used.

The organ should be obtained in very fresh state, freed of connective tissue and blood, and then divided into small portions which are ground into as fine a pulp as possible. This pulp is placed in a cloth strainer and washed with running water for several hours, being squeezed out from time to time until it is free of everything except parenchymatous tissue, and especially free of any trace of blood. The fat is next removed by treating with a slow current of carbon tetrachloride in a Soxhlet apparatus, until the liquid flows perfectly limpid and clear.

Finally the tissue is placed, portion by portion, into boiling water in order to remove any decomposition products of the albumins which it may contain. It is allowed to boil for 5 minutes and again put upon a filter where it is washed in at least 100 times as much distilled water as there was original tissue. The boiling and rinsing are repeated 5 to 10 times.

In order to be sure that nothing remains which might yield a false reaction, a control is carried out with *ninhydrin* in the following manner:

Two grams of pulp, for example, are boiled with 10 cc. of distilled water for 5 minutes in a test tube, and then put upon a small filter. To 5 cc. of the filtrate is added 1 cc. of a 1 per cent solution of ninhydrin, after which the boiling is repeated for a few moments. If a violet color does not appear, it means that the preparation is good. If the contrary is the case, the boiling in water and the successive washings must be continued.

Thus 200 gms. of organ produce scarcely two grams of product which can be used. The latter is immersed in a small flask containing boiled distilled water and covered with a layer of toluol to prevent evaporation and bacterial contamination.

The dialyzers should be carefully controlled as to their absolute impermeability for albumin and their permeability for the peptones. This control may be effected either with a solution of egg white or,

more surely, with a 5 per cent solution of casein; the least trace of which passing through the membrane can be detected with sulphuric acid, which produces a clouding (Swart and Terwen).[17]

For testing the permeability for peptones a solution of silk peptone (peptone-sericin) is employed. The dialyzing shell having been placed in an *Erlenmeyer* flask containing 20 cc. of distilled water, 2.5 centigrams of the 1 per cent solution of silk peptone are introduced. The contents both of the dialyzer and of the flask are covered with a fairly deep layer of toluol and the apparatus placed in the incubator for 16 to 20 hours.

At the end of this time 10 cc. of the dialysate are transferred with a pipette to a test tube and 0.2 cc. of a sterile freshly prepared 1 per cent solution of *ninhydrin* added. When boiled for one minute, with shaking, a violet color appears if dialysis of the peptone has gone on properly.

The dialyzers having been thus tested one by one, they are quickly passed through boiling water and preserved in distilled water beneath a layer of toluol.

Technique of the reaction. Having placed the dialyzer in an *Erlenmeyer* flask, one introduces into the former, with forceps, about 1 gram of the organ to be studied and then, with a pipette, 1 to 1.5 cc. of serum. 20 cc. of sterile distilled water are poured quickly into the flask. Both the inside and outside liquids are covered with a layer of toluol and the whole placed in the incubator at 37°C.

A control dialyzer should contain only the serum.

After 16 to 20 hours the *ninhydrin* reaction is performed with the dialysate, of which about 10 cc. are used.

2. The optical method

This second method is based upon the variations in deviation observed in the polarimeter with a solution of albumin when its molecules are broken down into peptones and amino-acids under the influence of cellular ferments contained in a pathological serum. This decomposition does not take place in the presence of a normal serum.

The method requires a special polarimeter, so that it is not practical except in laboratories particularly equipped for it.

[17] München. med. Wchnschr., 1914, 61, 603.

3. Application of the Abderhalden reaction to the diagnosis of tuberculosis

The reaction was applied for the first time by Abderhalden and Andryewsky[18] with bacterial bodies killed by heat and rid of fat and with a bacillary peptone prepared at the Rockefeller Institute in New York. It was found in this manner that the serum of tuberculous cattle attacks the *bovine* bacillus only, being inert when combined with the *human* bacillus.

Tuberculous tissue and the serum of tuberculous animals are said in all cases to give positive reactions and there should even be a special chemical specificity as regards the different lesions, since the serum of subjects with miliary tuberculosis leaves caseated lung intact, while attacking the tissue of caseous pneumonia.

Lampé and Ed. Arno, Fraenkel and Gumpertz[19] obtain altogether discordant results. Jessen [20] finds that the reactions are, in general, positive in tuberculous cases, except in the period of cachexia, during which ferments are not produced, and a study of the serum against the different organs enables him to determine the localization of the lesions. According to Krim,[21] the sera of certain tuberculous cases react not only with the tubercle bacillus but also with diphtheria bacilli and with placenta.

With a peptone which Flexner obtained from tubercle bacilli, Abderhalden[22] is said to have had quite accurate results by means of his optical method. He mixes this peptone with a tuberculous cattle serum in the tube of a polarimeter graduated in hundredths of a degree and, after having maintained the tube at a constant temperature, he observes a deviation which, in the case of a positive reaction, may reach as much as twenty hundredths of a degree.

The dialysis procedure, according to this scientist, enables him to prove that the sera of tuberculous cattle splits the cheesy matter of a fragment of caseous pneumonia. Ordinary bovine tuberculosis could thus be determined with certainty, but not the miliary infection, for which the test is negative once in five times.

[18] München. med. Wchnschr., 1913, **60**, 1641.

[19] Deutsch. med. Wchnschr., 1913, **39**, 1585; 1914, **40**, 589.

[20] Congr. des naturalistes et médicins allemands, Vienna, Sept., 1913:—Med. Klin., 1913, **9**, 1760.

[21] Russk. Vrach., 1913, **12**, 1502.

[22] München. med. Wchnschr., 1913, **60**, 1641.

Gwerder and Melikjanz[23] published a number of facts which tend to show that the serum of phthisical patients reacts with tuberculous lung in 93 per cent of cases, with the normal lung in only 69 per cent, and in 50 per cent with the normal liver.

It seems therefore that the specificity of the Abderhalden reaction is far from being as definite as might be wished and that it is hardly possible at the present time to yield any really useful information as regards diagnosis. The study, however, is still only in its inception and it is entirely possible that it will some day become a valuable means of determining the effects of a rational therapeusis in tubercle bacillus infection.

C. THE REACTION OF ACTIVATION OF COBRA VENOM

Quantity of free lipoids in the serum

In 1902 I pointed out[24] that when one places together *in vitro* cobra venom and erythrocytes of cattle, horse, rabbit, man, etc., freed of serum by several washings in physiological salt solution and successive centrifugations, one finds no hemolysis, whereas the latter manifests itself in a few minutes if there be added to the mixture a little horse or dog serum previously heated to 58°C.

Later studies by P. Kyes[25] and Hans Sachs, and then those of H. Noguchi[26] have shown that only sera which contain lecithin, fatty acids, or soaps, are capable of activating venom, that is to say of rendering it hemolytic; and that the activating action of fatty acids and soaps is prevented by the addition of a suitable amount of calcium chloride to the serum, while that of the lecithin is not so prevented.

Having found in 1908,[27] with L. Massol and M. Breton, that tubercle bacilli possess a very special affinity for lecithin and that the sera of tuberculous individuals, when deprived of complement by heating to 58°C., activate cobra venom, in other words render it hemolytic *in vitro*, while the sera of healthy subjects are incapable of accomplishing this, I thought that advantage might somehow be taken of this property to confirm or disprove a diagnosis of tuberculous infection.

[23] München. med. Wchnschr., 1914, **61**, 980.
[24] Compt. rend. Acad. des sci., 1902, **134**, 1446.
[25] Berl. klin. Wchnschr., 1902, **39**, 886; 918; 1903, **40**, 21; 57; 82; 956; 982.
[26] J. Exper. Med., 1907, **9**, 436.
[27] Compt. rend. Soc. de biol., 1908, **146**, 676.

The affinity of tubercle bacilli for lecithin is obvious from the following experiment:

Into a series of test tubes A, A′, A″, etc., is put 1 cc. of a suspension of fresh tubercle bacilli (bovine origin, corresponding to 0.5 per cent of dry bacilli by weight) with varying amounts of lecithin (0.4 cc. to 1 cc. of a 1 in 10,000 solution[28]). They are left in contact for two hours in the incubator at 37°C. and there are then added to each tube 1 cc. of a 5 per cent suspension of washed red blood cells (horse) and 0.5 cc. of a 1 in 5000 solution of cobra venom. Control tubes B, B′, B″ etc., receive the same quantities of lecithin + red cells + venom. Other control tubes C, C′, C″, receive tubercle bacilli + red cells + venom without lecithin.

In less than 30 minutes hemolysis is complete in all the tubes of the B series. It is absent, even after 18 hours, in the C tubes and likewise in the tubes of series A, where the tubercle bacilli had remained in contact with 0.4, 0.5, and 0.6 cc. of the lecithin solution. In the other tubes of series A, containing 0.7 cc. or more of lecithin, the red cells are hemolyzed.

The same experiment is repeated, replacing the fresh tubercle bacilli by dry bacilli, by bacilli heated to 120°C., by a 0.5 per cent solution of raw tuberculin (or of 5 per cent tuberculin precipitated in the cold with alcohol), or by the same solution sterilized at 120°C., and finally by culture broth without bacilli.

It is then found that the dry bacilli are quite as active with lecithin as fresh bacilli, but that on the contrary the bacilli sterilized at 120° lose almost entirely their initial avidity (hemolysis with 0.5 cc. of lecithin). The tuberculin prepared cold likewise deviates the lecithin and prevents it from acting upon the venom up to the maximum amount of 4 cc. (of 1 in 10,000 solution) for 1 cc. of solution of 5 per cent tuberculin. The same tuberculin sterilized at 120°C. is much less active and the culture broth without bacilli and without tuberculin is not at all so.

In the light of these results and of our earlier finding of the activating properties with respect to venom manifested by sera which

[28] The solution of lecithin is prepared by dissolving 1 gm. of lecithin in 100 gms. of pure methyl alcohol. One cubic centimeter of this dilution is added to 9 cc. of 0.85 per cent salt solution. A second dilution is made by adding 1 cc. of the preceding dilution to 9 cc. of saline. This last dilution of 1 in 10,000 is used for the reaction.

contain lipoids capable of activating venom (horse, dog, goat, rabbit, rat), we immediately thought of studying comparatively the behavior of the different sera of healthy animals or men and of tuberculous animals or men, either toward venom alone or after preliminary contact with a suspension of tubercle bacilli + venom.

For venom alone the activating property of lecithin-containing sera is very easily made clear by the following technique:

Into a series of test tubes is put 1 cc. of a 0.1 per cent solution of cobra venom (1 mgm.), freshly prepared, heated for a half hour at 75°C. and filtered through paper. To each tube is added 1 cc. of a 5 per cent suspension of horse red cells washed in physiological salt solution and centrifugated at least 3 times to remove all traces of serum.

Each tube afterward receives respectively 0.01 cc., 0.05 cc., 0.1 cc., 0.5 cc., and 1 cc. of the serum to be studied, previously heated for a half hour at 58°C. All tubes are made up to 3 cc. with physiological salt solution (0.85 per cent of NaCl). The tubes are incubated at 37°C. for a half hour after which the results are read. Hemolysis is the more complete the more there is of free lecithin in the serum employed, so that the method may serve to determine the approximate amount of lecithin which is contained in this serum.

Another series of control tubes all receive a uniform quantity of cobra venom (1 mgm.); a uniform quantity of washed erythrocytes and varying amounts, 0.05 cc., 0.08 cc., 0.1 cc. etc., of a 1 in 10,000 solution of pure lecithin prepared as stated above with physiological salt solution. All tubes are made up to 3 cc. and likewise put in the incubator. The results are read after a half hour.

If the tube of the first series, containing 0.1 cc. of the serum to be studied, shows complete hemolysis in the same time as the tube of the second series (containing 0.05 cc. of 1 in 10,000 solution of lecithin for example), it may be deduced that 1 cc. of serum contains the quantity of lecithin which is in 0.5 cc. of the 1 in 10,000 solution of lecithin, that is 0.00005 gm. of lecithin or other lipoids capable of activating venom. The part which lecithin plays in this activation can be determined by adding calcium chloride, which inhibits the activating action of fatty acids and soaps.

The series of experiments carried out as above has enabled us to establish the following facts:

1. Lecithin-containing sera, no matter whether the action of the

activating fatty acids has been destroyed by heating at 58° or by the addition of a sufficient quantity of calcium chloride, reveal the presence of this lecithin by the capacity which they confer upon cobra venom of hemolyzing washed red cells.

2. The approximate quantity of lecithin contained in the sera can be titrated by measuring the quantities of sera which are capable of activating a determined weight of venom.

3. The lecithin of activating sera can be deviated or fixed either by tubercle bacilli added in sufficient quantity, or by solutions of tuberculin prepared cold, so that when these sera have stood for a suitable time in the presence of bacilli or tuberculin, they lose the property of activating venom (5 mgm. of bacilli weighed dry can fix 0.0001 gm. of lecithin, that is 2 per cent of their weight).

4. The sera of *tuberculous* man or animals (not cachectic) contain a significant amount of lecithin, demonstrable by the foregoing reaction, whereas the sera of healthy men or animals of the same species do not contain it. In our experience, neither the serum of healthy new-born infants nor that of calves, after one hour of heating at 58°, is ever capable of activating venom. The same is true of sera of adult cattle which do not react to tuberculin, and for those of healthy men or swine. All of these sera, heated to 58°C., are *inactive.*

On the other hand, the sera of *tuberculous* men or cattle, similarly heated to 58°C. do activate venom, and the lecithin which they contain can be deviated *in vitro* by tubercle bacilli.

But the sera of the tuberculous are not alone in containing lecithin; those of normal subjects subjected to an anaesthetic such as chloroform or ether; those of syphilitics whether new-born or adults, and of patients suffering from cerebrospinal meningitis or Addison's disease, the sera of the insane, of general paralytics and perhaps of many sufferers from numerous troubles characterized by a more or less profound alteration of the nervous cells or suprarenal capsules, also contain it. They activate cobra venom as does the serum of tuberculous individuals. It seems, however, that the lecithin in these cases is in a different state, since it cannot be fixed by tubercle bacilli.

Perhaps the very evident affinity of tubercle bacilli and of tuberculin (prepared cold) for lecithin plays an important rôle in the general febrile reaction and in the local reactions of skin and mucous mem-

branes (cuti- or ophthalmo-reaction) which appear after subcutaneous injections or instillations of tuberculin upon mucous membranes. It is found in fact than when a solution of tuberculin has been left in the incubator for several hours in contact with a horse or dog serum previously heated for one hour at 58°C. and rich in lecithin, so that in the mixture there still remains after fixation an excess of lecithin capable of activating venom, the tuberculin thus treated is no longer capable of producing the ophthalmo-reaction. Its toxicity however does not seem diminished for tuberculous guinea pigs.

It may be asked whether it is not to this special affinity for lecithin on the part of the nervous cells that we must attribute the symptoms so characteristic of tuberculous meningitis and also the toxicity of tuberculin for healthy animals when this substance is introduced directly into the brain, while harmless for normal animals of the same kind when introduced subcutaneously, into the peritoneum, or intravenously.

Continuing our experiments, we were able to prove with L. Massol and C. Guérin[29] that certain animal species have a serum which constantly contains lecithin. In the following order they show a decreasing average richness in lecithin: the *horse, dog, rat, goat, sheep, rabbit.*

Now it is precisely *these species* which are the *hardest to tuberculize.*

The serum of the guinea pig on the contrary is very poor in lecithin; that of normal *swine, calves* and *cattle* and, as we have seen before, that of normal *new-born* infants and of *man never contain it. And these species are the most easily tuberculized.*

There is reason therefore to ask:

1. Whether the lecithin which constantly exists in the blood of certain normal animals is susceptible of being *fixed* or *deviated* by tubercle bacilli or by tuberculin prepared cold;

2. Whether artificial infection with tuberculosis or treatment with tuberculin of animals whose blood does not contain lecithin can cause the latter to appear in the serum.

In order to answer the first question, we combined in a series of test tubes, mixtures of 1 cc. of a 0.5 per cent suspension of tubercle bacilli (weighed dry) in physiological salt solution, plus the dose of serum to be tested which has shown itself capable of activating, in

[29] Compt. rend. Acad. des sci., 1908, **146**, 1076.

about 1 hour, 0.5 cc. of a 1 in 5000 solution of cobra venom; or else 1 cc. of a 0.5 per cent solution of tuberculin prepared cold with the same quantity of serum. The tubes were placed in the incubator at 37° for 2 hours, being shaken from time to time. To each of them were then added red cells and venom as in the activation experiments and the hemolysis taking place at room temperature, after periods of time varying from 1 to 6 hours was noted.

In this manner we found that in all cases the lecithin normally contained in the serum of the horse, dog, etc., was fixable by tubercle bacilli and by tuberculin.

The answer to the second question is furnished by the following experiment:

An 18 months old Flemish heifer, which does not react to tuberculin and whose serum does not activate cobra venom, receives, on the 9th of April 1908, 5 mgms. of a culture of bovine bacilli (origin: milk, of Nocard) into the jugular vein. On the second day after injection her temperature rises and oscillates between 39° and 40.4°. On the second and fifth days her serum does not contain lecithin. On the ninth day the temperature goes down and remains until the first of May between 38.5° and 39°, and throughout this period the serum is acquiring activating properties. Beginning with the second of May the temperature rises again and the serum ceases to activate. On May 8 there is a new fall of temperature coinciding with the reappearance of lecithin in the blood. On May 15 the fever definitely establishes itself at about 40°. The animal shows all the signs of an acute miliary infection and the serum is no longer active.

A Brittany heifer of 2 years, negative to tuberculin is injected subcutaneously on February 17, 1908, and is bled on April 11. Its serum does not activate cobra venom. On the same day there is injected into the jugular vein 0.5 gram of dry tuberculin prepared cold, dissolved in 20 cc. of physiological salt solution. There is no elevation of temperature thereafter, and the serum remains inactive during the succeeding days. On the 15th of April (4 days later) she is injected again into the jugular vein with 0.5 cc. of the same tuberculin. After 4 hours the temperature rises from 38.6° to 40.2° and at the end of 12 hours returns to normal. On the second day following, i.e., April 17, the serum contains lecithin and remains very activating until April 25. From this date on the lecithin disappears.

It is evident, therefore:

1. That experimental tuberculous infection carried out intravenously induces a discharge of lecithin into the serum each time that the temperature falls and that the lecithin disappears during the febrile periods;

2. That the intravenous injection of tuberculin into a healthy cow, repeated at a 5 day interval, gives the same result. After the second injection the animal reacts as if it were tuberculous; her serum becomes strongly activating for one week and then returns to normal.

Study of the blood of other tuberculous and healthy cattle enables us to assert that when these animals have tuberculous lesions without fever, their serum contains a lecithin-like substance which is capable of activating cobra venom; this substance seems to be more abundant as the lesions are more extensive. On the other hand, it disappears entirely when the cattle have fever and are cachectic. It is not present in the blood of healthy cattle.

Since calling attention to these curious reactions we have studied[30] them systematically in the sera of many subjects, normal or suspected of tuberculosis, or clinically tuberculous (man and cattle), and in the milk of both women and cows.

Of 77 sera of human tuberculous cases, the reaction of activation was found positive in the following proportion:

	per cent
Tuberculous cases in the first stage (according to Turban)	76
Tuberculous cases in the second stage	57
Tuberculous cases in the third stage	70

Among 26 subjects clinically non-tuberculous, the reaction of activation was positive 8 times (30.7 per cent).

The milk of 24 women chosen at random at a clinic for nursing infants was tested in the same way.

Each specimen (about 10 cc.) was coagulated with rennet. The whey, poured off and heated at 58°C. for a half hour, served for the experiments. One cubic centimeter of whey was mixed with 0.1 mgm. of venom and 1 cc. of a 5 per cent suspension of horse erythrocytes. The results were noted after 2 and 24 hours at laboratory temperature.

Of the 24 milks, 12 showed themselves to be activating for venom and 12 non-activating. The 24 women were tested with tuberculin

[30] Compt. rend. Soc. de biol., 1908, **65**, 648.

by the cuti-reaction. Of the 12 whose milk was activating, 9 gave a positive tuberculin reaction and 3 a negative reaction. Of the 12 whose milk was non-activating for venom, only one gave a positive cuti-reaction.

The same experiment carried out upon the milk of 8 healthy cows which had not reacted to tuberculin, showed but a single positive reaction of activation.

Neubauer and Seiffert,[31] repeating our work, confirmed the value of the reaction of activation in cattle experimentally tuberculized.

Neisser found likewise that, in the guinea pig, an increase in the proportion of serum lecithin coincided always with the presence of tuberculous lesions.

But there seems to be no doubt that, from the clinical standpoint, the fact that subjects suffering from a variety of diseases, particularly those involving the organs rich in lecithin (nervous system, suprarenal capsules), may have an activating serum, robs this reaction of much of its interest. This at any rate is the opinion of S. Pekanovich,[32] Fornario,[33] J. Nowaczinski,[34] and of Beyer.[35] It cannot be regarded as specific. If positive it is not proof of the existence of tuberculosis. But, by way of compensation, if it is negative in a suspected individual there is reason to believe that tubercle bacillus infection is excluded.

All in all, the activation of cobra venom by serum indicates simply a discharge of lecithin or other analogous lipoids into the circulation and we know today that in tuberculous infection, where this discharge is manifestly frequent, it appears associated with lesions of the *suprarenal capsules*, lesions which moreover are reproduced by experimental tuberculin intoxication.

D. THE POTASSIUM IODIDE REACTION

Sticker proposed (1891) to use iodide of potassium to give a diagnostic reaction in tuberculous cases. This author injected the compound subcutaneously in a dose of 0.5 to 1 gm. in order to bring about the appearance of moist râles, and of bacilli in the sputum.

[31] Ztschr. f. Fleisch.- u. Milchhyg., 1909, **19**, 193.
[32] Deutsch. med. Wchnschr., 1910, **36**, 162.
[33] Roy. Acad. de Med. di Torino, 1910, Nos. 5, 7.
[34] Ztschr. f. Tuberk., 1911/12, **18**, 26.
[35] Ibid., 1910, **16**, 485.

Rondot, Vetlesen, and afterward Wells[36] confirmed these facts after trials on a certain number of patients. If properly administered, iodide of potassium is said to produce only a temporary reaction with no harmful effects (Landouzy).

The action of potassium iodide has been studied experimentally by F. Sorrel[37] in the tuberculous guinea pig.

This animal, 4 weeks after being tuberculized, gives a temperature reaction 7 to 8 hours after subcutaneous injection of 10 centigrams of iodide of potassium. The thermic reaction is no stronger if 20 to 25 cgm. are injected. When the injections of iodide are repeated, stronger reactions are obtained than at first. The non-tuberculous control animals maintain their normal temperature.

By injecting daily for two weeks a dose of 10 centigrams of iodide, Sorel succeeded in rendering his guinea pigs non-sensitive to this substance, just as one can bring them to tolerate with impunity several centigrams of tuberculin. Tuberculous guinea pigs habituated to the iodide reacted to tuberculin and vice versa. One is therefore justified in regarding the two sorts of reaction as different. In fact, an intraperitoneal injection of iodide provokes in a tuberculous guinea pig an exudate which does not contain tuberculin and the injection of 0.25 cc. of this exudate into the brain of a tuberculous guinea pig is without effect. It is therefore probable that the iodide does not set free any tuberculin in the body of tuberculous animals.

This reaction is moreover non-specific. Marchoux and Bourret have pointed this out in *leprosy cases.* It is known that it is produced also in *actinomycosis, sporotrichosis,* etc. It is however of a certain biological interest and may be tried with advantage in some cases.

E. MIOSTAGMIN REACTION

There is a well known physical principle that *the number of drops furnished by a determined volume of liquid is in inverse ratio to the surface tension of this liquid.* On this principle has been constructed the *drop-counter* of Duclaux for determining quantities of alcohols and volatile acids and the *stalagmometer* of Traube which is applied to the study of the *viscosity* of different body fluids.

[36] J. Am. Med. Assn., 1899, **32**, 216.

[37] Compt. rend. Soc. de biol., 1909, **66**, 524;—Ann. de l'Inst. Pasteur, 1909, **23**, 533.

When the surface tension falls, the number of drops furnished by a given volume of liquid increases and the drops are then smaller. This fact makes possible the miostagmin reaction (from the greek: meïon, smaller, and stigmôs, falling drop).

Using the stalagmometer of Traube, Ascoli[38] found that the surface tension of a typhoid serum was lowered when a typhoid antigen was mixed with it.

Izar,[39] and later Roncaglio[40] have applied this reaction to the diagnosis of tuberculosis. By counting the number of drops furnished at room temperature by the serum to be studied, the latter being diluted 1 in 20, Izar finds for example 56 drops. Taking afterward 9 cc. of the dilution of serum and 1 cc. of freshly prepared antigen solution and leaving the mixture for one hour at 50°C., there is found for the same volume an increase which generally amounts to 1 or $1\frac{1}{2}$ drops.

S. Wyschelessky[41] employed Izar's technique varying the dilutions of antigen with normal sera and with tuberculous human or beef sera. Eight tuberculous sera gave him an average increase of 2.18 *drops* and seven normal sera an average increase of 1.96 *drops*. After mixing with the antigen the difference is therefore only 2.18 *drops* minus 1.96 = 0.22 *drops* in favor of the tuberculous sera. This difference is so slight and so inconstant that its use for the diagnosis of tuberculous infection does not seem possible.

F. HYPOPHYSIS TEST

H. Claude, A. Baudoin, and R. Porak[42] showed that the injection of an extract of the posterior lobe of the hypophysis sets up a temporary glycosuria which is much accentuated in arthritics. On the contrary, in tuberculous subjects and in rabbits experimentally tuberculized, this glycosuria either does not occur or is only very mild, even though one injects a dose of the extract corresponding to the whole of one lobe of the gland.

[38] München. med. Wchnschr., 1910, **57**, 62; 403.
[39] Ibid., 1910, **57**, 182; 403; 1170; 2129.
[40] Clin. vet. (Milano), 1912, **35**, 633.
[41] Ztschr. f. Tuberk., 1912, **21**, 209.
[42] Compt. rend. Soc. de biol., 1917, **74**, 529.

G. URINARY ELIMINATIONS AND DIAGNOSTIC REACTIONS OF URINE IN TUBERCULOUS INFECTION

At the beginning of a tuberculous infection the clinical study and chemical analysis of urine give but very little useful diagnostic information. Various clinicians (Landouzy, Léon Bernard, Labbé and Castaigne) have noted however the frequent existence of polyuria and a mild so-called pretuberculous albuminuria which, according to P. Teissier, bears a close relationship to that in the congenital mitral stenosis described by this author as a form of hereditary tuberculosis but which, in the opinion of Talamon, is due rather to the vaso-dilator action of the tuberculous poisons upon the vessels of the kidney. This albuminuria is also observed in experimental tuberculosis (Ed. Dehaussy).

The chief interest lies in variations in the mineral elements since they are, in a certain measure, indicators of the defensive reactions of the body. Albert Robin has studied this phase considerably. He, as well as Darenberg, has called attention to the important losses in phosphates and calcium undergone by tuberculous cases in process of evolution. These losses can be calculated by measuring the coefficient of demineralization, in other words the relationship of mineral matter to the fixed residue (a relationship for which the normal value is 22 to 35 per cent); that of phosphates to urea (normally 8 to 11 per cent) and that of phosphates to total nitrogen (normally 18 per cent).

It appears meanwhile from recent investigations on the part of various clinicians, particularly of H. Labbé and G. Vitry, that these figures are reached only exceptionally and that the phosphaturia claimed for the tuberculous is far from frequent.

In any event it should be realized that observations up to now by means of the various methods based upon study of the urinary excretions, of the balance of *ingesta* and *excreta*, as well as by calcination, have furnished but very little exact information.

Servonat and Rebattu[43] have approached the question experimentally. They chose the guinea pig because of the possibility of calcining the whole of the animal and of carrying out analyses on the skeleton on the one hand and of the whole of the soft parts on the other.

[43] J. de physiol. et de path. gén., 1919, **18**, 934.

Their experiments indicate that the mineral composition of the body is but very little modified by tuberculosis. The variations in phosphoric acid are very irregular. On the other hand, there is a lowering of the calcium content of the soft parts and above all of the skeleton. It is this fact which stands out the most prominently. The much more marked decalcification of the skeleton is said to be due to the fact that therein lies the mineral reserve on which the body draws according to its needs.

This decalcification may result from the fact that the tubercle bacillus produces acids in the body as well as in cultures on artificial media.

In the early stage of tuberculosis the urinary acidity is almost constantly normal or slightly increased; afterward it soon undergoes a marked fall which has been noted by many observers (Grimbert and Morel,[44] Olivesco, Le Coat de Kerveguen, Nicolaidi, Chatelain, Pertik,[45] H. Labbé and G. Vitry[46]).

Ed. Dehaussy,[47] in my laboratory, took up the experimental study of this question by observing variations in chlorides, phosphates, uric acid, total nitrogen and calcium in rabbits maintained in a state of nutritional equilibrium. He could usually prove the existence of an excess of chlorides from the beginning of the infection; but on the approach of death the chloride content fell from 0.44 gm. in 24 hours to 0.036 gm. In the normal control rabbits, under the same regime, the chloride output was maintained between 0.17 and 0.2 gm.

The amount of phosphates eliminated is also much increased 2 weeks after intravenous virulent inoculation (1 mgm. of bovine bacilli). From 0.15 to 0.175 gm. in the normal rabbit, it passes to 0.6 gm. in the tuberculous. Ten days later, the animal does not excrete more than 0.07 gm., which figure rises slightly at the moment of death.

Uric acid excretion, at first a little increased in the early stage, afterward falls off progressively. In the normal subject it oscillates from 0.01 to 0.014 gm.; in the early stage tuberculous cases it stands at 0.038 gm.; there are then found successively 0.018, 0.01 and finally 0.008 gm.

[44] Compt. rend. Soc. de biol., 1912, **72**, 179.
[45] Virchow's Arch., 1913, **213**, 465.
[46] Presse méd., 1914, **22**, 437.
[47] Compt. rend. Soc. de biol., 1914, **77**, 124.

The same is true of total nitrogen which goes from 0.9 to 1.74. With the disease at its height there is found from 0.54 gm. to 0.658 gm.; then 4 and 3 days before death, 1.35 gm. The normal rabbit eliminates from 0.849 to 0.902 gm.

As regards calcium, an intense calciuria is constantly observed in the beginning. From a level of 0.0004 to 0.0005 gm. in the normal rabbit, the urinary output of this substance passes to 0.0018, 0.003, 0.0037, and 0.0047 gm., to redescend to 0.0029, and 0.0016 gm., then finally to 0.002 gm., at death.

H. Labbé and Mlle. Golgofsky,[48] and M. Gerard[49] have studied the urinary elimination of saponifiable and non-saponifiable substances in tuberculous cases according to a technique suggested by the method of Neubauer. They came to the conclusion that, in normal subjects, the quantity of total organic substance extractible by ether (acids, saponifiable and non-saponifiable substances) is exceedingly small and varies but little, while in tuberculous subjects the amount is on the average twice as great. The non-saponifiable substances thus eliminated are made up in large part of cholesterol and the acids, especially hippuric acid.

1. Uro-reaction of Malmejac

The reduction of mineral elements in the tuberculous is said to result from the accumulation of an excess of carbon dioxide in the blood. The later is said to manifest itself in a remarkable persistence of urinary acidity which Malmejac[50] proposed to utilize for the early diagnosis of tuberculous infection.

According to this author, if urines of tuberculous individuals who do not receive medication are collected in sterile bottles and kept in free contact with air, but protected from dust by means of a simple paper covering, they preserve their acid reactions for periods varying from 12 days to 3 months and more, whereas urines of healthy individuals become alkaline spontaneously, under like conditions, in from 3 to 10 days.

Furthermore, if the daily urinary acidity of tuberculous cases is charted, as well as that of healthy individuals, it will be noted, according to Malmejac, that the former preserve their initial acidity

[48] Compt. rend. Soc. de biol., 1912, **73**, 221; 332.
[49] Internat. Congr. on Tuberculosis, 10th., Rome, 1912.
[50] Presse méd., 1909, ii, 665.

for some time and, as a result, show a long plateau at the beginning. But this does not hold true for the urine of the non-tuberculous; here the plateau does not exist, the acidity curve falling from the second day.

The persistence of this acidity of the urine on the part of the tuberculous is said to be prolonged in proportion to the progress of the disease. The early plateau should have an average of:

17 days in the first stage (Turban);
26 days in the second stage;
40 days in the third stage.

The degree of acidity, it is said, varies in the same way. One should find a hypo-acidity in the two first stages of the disease and a very marked hyperacidity in the third. Malmejac gives the following average figures calculated in H_2SO_4 per liter,—the urinary acidity of normal man averaging 1.35 grams per liter:

0.6756 gm. in the first stage;
0.991 gm. in the second stage;
2.287 gm. in the third stage.

There is not necessarily any relation between the degree of acidity and its persistence.

The technique for determining urinary acidity quantitatively is the following:

Ten cubic centimeters of urine collected and preserved as described above are measured out with a pipette, and transferred to a flat-bottomed vessel. In order to lessen the color, 50 cc. of distilled water are added, and afterwards 4 to 5 drops of 1 per cent solution of phenolphthalein. The solution is then very exactly titrated with a decinormal solution of sodium hydroxide (1 cc. of this solution corresponds to 0.0049 gm. of sulphuric acid) and the result expressed in sulphuric acid per liter.

The results of this "uro-reaction" have not been confirmed by the studies of Chatelain,[51] nor by those carried out at the King Edward VII Sanatorium at Midhurst (Sussex)[52] nor by the work of R. Letulle[53] in my laboratory upon 100 urines of healthy, tuberculous or suspected persons, nor again by that of T. Pertik.[54]

[51] Thèse, Nancy, 1910.
[52] Fourth Ann. Rep., 1909/10.
[53] Thèse, Paris, 1912.
[54] Virchow's Arch., 1913, **212**, 465.

At the same time, Grimbert and Morel[55] obtained some interesting hints from it by adopting a technique which permits correction of the retarding effects of the ammoniacal salts and which corrects the error due to the presence of calcium salts. But in the present state of our knowledge it does not seem that the measure of the persistence or of the degree of urinary acidity can furnish any information really useful for the diagnosis of tuberculosis.

2. Reaction of Moriz-Weiss

In 1910, Moriz-Weiss[56] (of Vienna) showed that urines of tuberculous cases frequently contain a urinary pigment chromogen, *urochrome*, which can be demonstrated by means of potassium permanganate.

The technique of this author is very simple:

A first test tube is filled one-third full with the urine to be examined; this is diluted with twice its volume of water and mixed well. Half of the contents of the first tube are then poured into a second tube of the same size. Three drops of a fresh 0.1 per cent solution of potassium permanganate made *with distilled water* are then added to one of the tubes and mixed. If the reaction is positive, a yellow color appears which stands out distinctly in comparison with the control tube.

Only clear urines may be used for when the specimen is too albuminous or too heavily charged with urobilin or bile pigments the specific coloration is not readily apparent. The *urochrome* which is an oxidation product of *urochromogen* is, according to Moriz-Weiss, the same body (proteic acid) which gives the diazo-reaction of Ehrlich. Its presence should indicate a more or less rapid breaking down of the tissues of the body.

Vitry,[57] Mladenoff,[58] Laignel-Lavastine and Grandjean,[59] and P. Merklen have studied the reaction of Moriz-Weiss and found it almost always positive in tuberculous cases with cavities and in

[55] Compt. rend. Soc. de biol., 1912, **72**, 179 (quoted by Labbé and Vitry; Compt. rend. Soc. de biol., 1913, **75**, 530).

[56] Med. klin., 1910, **6**, 1661;—München. med. Wchnschr., 1911, **58**, 1348.

[57] Compt. rend. Soc. de biol., 1912, **73**, 462;—Bull. Soc. d'étude scient. sur la tuberc., 1913, Jan.;—Rev. de la tuberc., 1914, 2. s., **11**, 177.

[58] Thèse, Paris, 1912.

[59] Bull. et mém. Soc. méd. d. hôp. de Par., 1913, 3. s., **35**, 1253.

the acute tuberculoses; negative in benign forms and in the early stage of the disease. It is constantly negative also in experimental tuberculosis of the rabbit (Ed. Dehaussy). But it is positive in many other severe infections, in typhoid fever, erysipelas, measles, under the influence of certain medicaments (cryogenin), and even now and then in healthy individuals, according to Dufour and Thiers,[60] Pierret and Leroy, Pierret and Bardou.[61] These facts detract considerably from the value attributed to it. Yet it seems that, in tuberculosis, it is an unfavorable prognostic sign when, followed day by day in the same subject, it gives a constantly positive result (Tecon and Aimard,[62] P. Courmont,[63] Dorge[64]).

3. Examination of urine for tuberculin

Some authors say that they have succeeded in demonstrating the presence of *tuberculin* in the urine of the tuberculous, either by inoculation into the tuberculous guinea pig (Rappin and Fortineau) or by the reaction of Bordet-Gengou using the urines as antigen (Marmorek, Bergeron, Robert Debré and Paraf).[65] This last reaction may be positive in some rare cases where pyuria exists, but it is then the *bacilli* expelled in abundance which play the rôle of antigen, and not the *tuberculin*. It has been in no wise demonstrated that the latter can be found in a free state in urine, nor indeed in the blood of patients.

[60] Bull. Soc. de pédiat. de Par., 1913, **15**, 274.
[61] Echo méd. du nord., 1913, **17**, 173; 245.
[62] Paris méd., 1912/13, **11**, 577.
[63] Soc. de méd. des hôp. de Lyon, 1913, **11**, 546.
[64] Thèse, Lille, 1913.
[65] Compt. rend. Soc. de biol., 1911, **71**, 65; 169; 228.

CHAPTER XXXV

PHYSIOLOGICAL ACTION OF TUBERCULINS.—MECHANISM OF TUBERCULIN REACTIONS

A. COMPARATIVE TOXICITY OF TUBERCULINS FOR NORMAL AND TUBERCULOUS SUBJECTS

We have already studied—in Chapter V, which treats of *endo* and *exobacillary* tuberculous toxins—the manner of preparation of the various tuberculins and the circumstances which led to their discovery. Therefore we shall here recall only that these substances are relatively little toxic for healthy animals while extremely poisonous for the tuberculous.

Only very active tuberculins like *Tuberculol B and C* of Landmann, employed in large doses (respectively 0.5 cc. and 1 cc.), are capable of causing death in healthy guinea pigs (P. Geibel).[1] To obtain the same effects, one must use about 1 gm. of raw tuberculin of Koch precipitated with alcohol, and about 0.3 gm. in the mouse. Consequently it was asked whether the phenomena of intoxication were not simply due to the peptones which these products contain in abundance. A. Marie and M. Tiffeneau[2] settled the question by using tuberculin without peptone and adopting the intracerebral method of inoculation recommended by Von Lingelsheim and by A. Borrel, the technique of which I have already described. It was thus demonstrated that 3 to 4 milligrams of ordinary precipitated tuberculin, and only 0.75 milligrams of precipitated peptone-free tuberculin suffice to kill a normal 500 gm. guinea pig when the poison is brought into direct contact with the cerebral cells.

On the other hand, these same tuberculins, introduced by subcutaneous inoculation, and above all by intracerebral injection into tuberculous animals, exhibit an infinitely greater toxicity.

[1] Ztschr. f. Hyg., 1913, **73**, 13.

[2] Compt. rend. Soc. de biol., 1909, **66**, 206.

This will be seen in the figures here given (Marie and Tiffeneau), which are of value only when studied comparatively:

	GUINEA-PIG WITH TUBERCULOSIS OF 3 TO 4 WEEKS STANDING	
	Subcutaneous injection	Intracerebral injection
Raw tuberculin of Koch	0.075 cc.	
Precipitated tuberculin	0.03 gm.	0.00001 gm.
Precipitated tuberculin, purified	0.005 gm.	0.00075 gm.

According to H. Bing and V. Ellermann,[3] certain phosphatids have the property of increasing the toxic effects of tuberculin upon the tuberculous organism. This *activating* property is manifested particularly by a diamidophosphatid of egg yolk, *albine*. The lecithin, cephalin, cholesterol, oleic acid, sodium oleate and the other lipoids of egg yolk or of sera do not exert the same action.

Many workers, and in particular recently W. G. Ruppel and K. Joseph,[4] have studied the comparative action of different tuberculins upon healthy and tuberculous animals. It is proved that, for the tuberculous guinea pig, the toxic dose of substances extractible with water from ground up bacilli is about 500 times greater than for the healthy guinea pig; and that, on the other hand, the filtrate from cultures (such as that employed in the preparation of the tuberculin of Denys, of Louvain) contains nothing toxic for the normal animal—except the glycerin which alone kills the guinea pig in a dose of 4 cc. when pure, and in smaller dose when mixed with impurities derived from broths.

Ruppel and Joseph, and Geibel,[5] have also found that, as Von Behring had already observed, the nucleic acid derived from tubercle bacilli (tuberculinic acid) is 100 times more toxic for the tuberculous than for the normal guinea pig, while the nucleic acid derived from the thymus has the same toxicity (100 times less than that of tuberculinic acid) for the healthy animal as for the tuberculous.

It seems indeed that in man, in the absence of any tuberculous infection, subcutaneous injection,—or absorption in any other way—

[3] Biochem. Ztschr., 1912, **42**, 289.

[4] Ztschr. f. Immunitätsforsch., 1914, **21**, 277.

[5] Ztschr. f. Hyg., 1913, **73**, 13.

of even considerable quantities of tuberculin, is entirely innocuous. On the other hand minimal doses,—such as one-thousandth of a milligram, at times even one-ten-thousandth of a milligram of Koch's old tuberculin,—injected subcutaneously, produce in tuberculous individuals a febrile reaction which is always evident and relatively intense.

It is important to know that the activity of tuberculins varies with the derivation of the bacilli. Human tuberculin best reveals tuberculoses in mammals, in cattle as well as in man, but it is not suitable for disclosing avian tuberculosis, except in swine. Bovine tuberculin, even in cattle, gives a weaker reaction than human tuberculin. Avian tuberculin is very effective in disclosing tuberculosis in birds and, in swine infected with avian tuberculosis, it gives reactions which are much stronger (particularly the local reactions, intradermic reactions, e.g.,) than with the tuberculin from mammalian types (Bang).[6]

B. RELATIONSHIP BETWEEN SUSCEPTIBILITY TO TUBERCULIN AND THE DEGREE OF INFECTION

Marmorek[7] showed that there need not necessarily exist follicular lesions for one to observe extreme susceptibility on the part of the tuberculous body to tuberculin, since if a small quantity of virulent bacilli be injected subcutaneously into a guinea pig and 15 to 20 minutes later 0.3 cc. of raw tuberculin (a harmless dose for normal animals), the temperature is seen to rise in as few as 3 to 5 hours to 40° and above. But, if one waits longer than one hour and a half after the virulent inoculation before injecting the tuberculin the latter no longer causes a rise of temperature,—probably because the bacilli have all been phagocytized with the result that the tuberculin does not reach them.

Later, on the contrary, when follicular lesions are formed or when a bacillemia exists, the thermal reactions appear anew and with a small dose of tuberculin are usually the more intense the more limited the infection. Yet, as the disease extends, the dose of tuberculin necessary to induce a febrile reaction in man, or death in experimental animals, becomes smaller and smaller. Thus A. Borrel has noted

[6] Kgl. vetarinarog. landbokogskole Aarskrift, 1917.

[7] Compt. rend. Soc. de biol., 1903, **55**, 1650.

that while on the twelfth day after infection 0.1 milligram of precipitated tuberculin kills the guinea pig by the intracerebal route, 0.001 mgm. of the same tuberculin is sufficient to kill under the same conditions a guinea pig infected 30 days before, and only 0.0001 mgm. the pig infected 40 days before.

Susceptibility to tuberculin appears after one or two inoculations of bacilli, even though killed with heat (Borrel, A. Sata and Wolff-Eisner) and manifests itself as early as a few hours after the intraperitoneal injection of normal guinea pigs with 6 to 8 cc. of a paste of tuberculous organs (liver, spleen or glands (O. Bail).[8]

But it is important to observe that the later injection of tuberculin is not necessary to cause death of the animals under these circumstances. F. Neufeld and H. Dold[9] have in fact demonstrated that rabbits and guinea pigs injected intraperitoneally, either with a suspension of the spleen or glands of tuberculous guinea pigs, or with a few cubic centimeters of a paste prepared from lesions of tuberculous cattle, die in 1 to 5 days.

Tuberculous organs from the same species of animal are more toxic than are those taken from another species. But whatever their origin, they set up locally very violent inflammatory lesions whose cause escapes us and which are never observed after intraperitoneal injection of a paste of normal organs. It may be that the normal body fluids or the macrophage cells react upon the tuberculous cells to liberate a particularly active tuberculous poison.

C. MECHANISM OF THE SPECIFIC ACTION OF TUBERCULINS.—ITS RELATIONSHIP TO THE PHENOMENON OF ANAPHYLAXIS AND TO ANAPHYLATOXIN

From the time that Robert Koch acquainted us with the extraordinary susceptibility of tuberculous animals and patients to tuberculin, many hypotheses have been advanced to explain the phenomenon. At first it was thought that the febrile reaction resulted from the fact that tuberculin, introduced artificially, added itself to that which was already present in the infected organism. But this theory of "addition" became untenable when it was learned that tuberculin produced intense local reactions about old tubercu-

[8] Ztschr. f. Immunitätsforsch., 1909, **4**, 470; 1912, **12**, 451.

[9] Arb. a. d. k. Gsndhtsamte, 1912, **38**, 275.

lous foci which were latent and therefore incapable of forming abundant quantities of free tuberculin in the body fluids, and when it was also found that repeated injections of very small doses of tuberculin, powerless in themselves to produce perceptible thermal reactions, are nevertheless sufficient to induce inflammatory cellular reactions within and round about these same tuberculous foci.

Robert Koch used to think that tuberculin activated the process of necrosis of tuberculous foci, as well as the resulting fever of resorption.

In the opinion of Arloing, Rodet and J. Courmont, the hyperthermia was the result of the destruction of certain elements of the body which then acted upon the thermogenetic nervous centers.

Moussu[10] having established that cultures of tuberculosis enclosed within porous porcelain filters and introduced into the peritoneum of normal cattle behave as do tuberculous foci,—that is to say the animals react to tuberculin,—concluded therefrom that the reaction does not depend upon the presence of bacilli in the organism which reacts, but indeed upon an impregnation of the latter with toxic products elaborated by the bacilli.

A. Sata and Wolff-Eisner repeated the same experiments with identical results and S. Matsumura[11] saw that tubercle bacilli killed with heat, then enclosed in collodion sacs and placed in the peritoneum of normal guinea pigs, confer upon these animals, after 2 to 4 weeks, a tuberculin susceptibility which differs in no wise from that presented by other guinea pigs directly inoculated with cultures. But Trudeau, Baldwin and Kinghorn[12] do not accept these facts as proved, since when Berkefeld bougies or collodion sacs containing virulent bacilli have been left for 2 to 3 months in the peritoneal cavity of rabbits, the injection of tuberculin causes no thermal reaction and no local reaction about the sacs.

The same authors attempted to fix extracts of the bacilli to powdered animal charcoal, as I had formerly done with the *abrine of jequirity*,[13] and to inject suspensions of this substance under the skin or into the peritoneum of guinea pigs. The carbon being insoluble, it was hoped that deposits of bacillary proteins would be

[10] Compt. rend. Soc. de biol., 1905, **59**, 409; 463.
[11] Ztschr. f. Immunitätsforsch., 1914, **22**, 535.
[12] J. Med. Research, 1904, **12**, 169.
[13] Ann. de l'Inst. Pasteur 1896, **10**, 675.

formed in the tissues and diffuse themselves slowly, as in the case of a true tubercle. But the animals thus treated never reacted to tuberculin when tested after intervals varying from 21 to 261 days. They were subsequently infected with virulent bacilli and became tuberculous in the same time as the controls.

The discovery of "sensibilisatrices" or antibodies whose presence can be detected in the serum of tuberculous individuals by the complement fixation test of Bordet-Gengou, employing the bacilli or tuberculins as antigens, soon led Wassermann and Bruck[14] to propose an interpretation of the tuberculin reaction which at first seemed very inviting. These scientists thought that in and about the tuberculous foci there is formed an antituberculin capable of fixing the tuberculin. This antituberculin, produced in excess at the site of the tuberculous lesions, might even be diffused in the organism and be recovered in greater or lesser quantity, free in the blood. The general thermic reaction and the focal reactions were regarded as the result of the combination or neutralization of this antituberculin by the tuberculin artificially introduced, so that the reaction of complement fixation takes place thus *in vivo* and *with most intensity at the site of the tuberculous lesions.*

Since healthy subjects produce no antituberculin in the body fluids, it is understood why injections of tuberculin do not call forth in them the formation of any temperature raising complex.

The term antituberculin is obviously badly chosen, since it gives the idea that one is dealing with an "antitoxin" capable of neutralizing tuberculin in a manner analogous to that which governs the neutralization of diphtheria or tetanus toxin, or venoms, by the corresponding antitoxic sera. Now, in the hypothesis of Wassermann and Bruck, the complex resultant from the combination of the antituberculin with the tuberculin is not precisely an antitoxin, in as much as it manifests itself by toxic effects of which the thermal and focal reactions are the expression.

The mechanism of tuberculin reactions is much better understood if we go back to facts studied by Maurice Nicolle[15] relative to his "general conception of antibodies," and demonstrating the existence of a *lysin of tuberculous endotoxin* in the humors of tuberculous individuals.

[14] Deutsch. med. Wchnschr., 1906, **32**, 448.
[15] Ann. de l'Inst. Pasteur, 1908, **22**, 132; 237.

This lysin breaks down the tuberculin injected and sets free the substance which determines local inflammatory and general febrile reactions.

In the local inflammation with the cuti- or ophthalmo-reaction, of which we shall speak, its effects are particularly to be observed.

It is only necessary to bring together *in vitro* the serum of a phthisical patient and tuberculin in proper proportions and to then instill the mixture upon the conjunctiva of healthy human beings or healthy animals. The lysin contained in the serum, in contact with the tuberculin, liberates the substance which causes the phenomenon of the ophthalmo-reaction. J. Hekman[16] has published some very interesting experiments bearing upon this subject.

To one part of 40 per cent tuberculin are added 9 parts of serum from a phthisical patient, which makes a 4 per cent tuberculin. They are allowed to act upon one another for 5 minutes at room temperature, after which 3 to 4 drops are instilled into the conjunctival sac of one eye of a normal rabbit or guinea pig. After 2 or 3 minutes the procedure is repeated. The instillations quickly induce a cloudy exudate containing some leucocytes and some mucus. The reaction lasts 5 to 10 minutes after the second instillation. Injection of the vessels is but little marked in the guinea pig; in the rabbit it is greater.

Since neither the non-instilled eye nor the control animals react to tuberculin or to phthisical serum alone, it becomes evident that a special combination is produced between the two substances. It is probable that this is effected particularly at the expense of the tuberculin which undergoes somewhat profound modifications, since if the mixture be left too long it becomes inactive.

If the serum is heated to 58°, the reaction is weaker but not abolished. With tuberculin precipitated with alcohol, reactions are still obtained, but are less marked.

In the animals used in these experiments, autopsy showed the absence of any tuberculous lesion. The sera of different tuberculous individuals do not act with the same intensity upon the tuberculin. The most intense reactions are obtained with tuberculoses in the early stage and particularly with miliary tuberculosis. Certain of the latter gave sera of which dilutions of 1 in 10 or 1 in 20 were

[16] Nederl. Tijdschr. v. Geneeskunde, 1913, Dec. 13 (Abstr. in Semaine méd., 1914, **34**, 165).

still active. The sera of chronic tuberculous cases act but weakly. One can obtain the reaction moreover with very fresh serous effusions or with the cerebrospinal fluid from tuberculous meningitis.

It is much more difficult to induce general reaction phenomena in normal animals by injecting them either intraperitoneally, or under the skin, or even into the veins, with mixtures of tuberculin and sera of tuberculous subjects. When the proportions of the components of the mixture are such that a sufficient quantity of tuberculin can undergo lytic decomposition unmistakable thermic reactions are observed which do not occur with normal serum, but they are always of short duration and can be detected only by frequently taking the temperature. These injections moreover do not provoke or induce any other functional trouble.

The theory of lytic action based upon the studies of M. Nicolle and his collaborators, has since been adopted by Wolff-Eisner.[17] It explains very well why the general tuberculin reactions are intense and appear quickly in subjects whose body fluids are rich in lysin; and if it is true that lysin accumulates or is produced in greater abundance about tuberculous foci *in evolution*, it explains why these foci, following an injection of tuberculin, become the seat of inflammatory processes so definitely localized.

It has been asked whether, after all, we are not dealing with a phenomenon analogous to the *phenomena of anaphylaxis* made known to us by Ch. Richet. It does not seem so. Despite what some authors have written, especially Gougerot,[18] it hardly appears that tuberculin can be regarded as a substance which is *anaphylactizing* in itself; that it exerts its specific action only upon products secreted *in vivo* by the tubercle bacillus, products found either fixed in the tissues or organs which are the seat of tuberculous lesions, or free in the body fluids, and principally in the blood of tuberculous subjects.

To be sure, one can sensitize normal animals with *preparatory* injections (especially intravenous) of large doses of tuberculin as Marie and Tiffeneau, Slatineanu and Danielopolu, O. Bail, Orsini and other investigators have shown. I succeeded myself, with M. Breton and Georges Petit,[19] in producing the ophthalmo-reaction

[17] *Früh diagnose und Tuberkulose-Immunität.* Wurzburg, 1909.
[18] J. méd. français, 1913, **6**, 19.
[19] Compt. rend. Soc. de biol., 1907, **63**, 296.

in rabbits 16 hours after an intravenous injection of tuberculin. But this sensitization disappears within a few days when the tuberculin has been eliminated, instead of persisting and increasing as would be the case if the tuberculin were exerting a true sensitizing action.

On the other hand, when *normal* men and animals are injected with fairly large and progressively increasing doses of raw tuberculin (in man 2 to 10 mgms. for example), febrile reactions are elicited after 7 to 8 injections. But these reactions, though often intense, are always of short duration and very premature and have not the same character as true tuberculin reactions. They are moreover inconstant and, if the injections are continued without increasing the dose, they become weaker and then disappear entirely instead of increasing, as would be the case if the tuberculin were producing a sensitization.

We were able moreover to convince ourselves that these pseudo-reactions in normal subjects, apparently sensitized, were due to impurities in the tuberculin and not to the tuberculin itself, inasmuch as the same experiments performed with a non-peptonized tuberculin prepared from cultures upon the succinimid-mineral medium adopted by us,[20] never led to the same result. Neither in normal animals nor human beings were we able to obtain sensitization.

We know that the *genuine anaphylaxis* can be transmitted passively to a normal animal by transfusion or even by the simple inoculation of a quantity of the blood of an animal rendered sensitive. It is true that several workers, particularly Yamanouchi,[21] Bauer,[22] and later Sata,[23] claim to have succeeded in demonstrating passive anaphylaxis in normal rabbits and guinea pigs into which they had injected the blood of tuberculous rabbits and human beings. O. Bail with 45 different specimens of tuberculous material claims to have obtained 45 positive reactions.

By injecting normal animals several times with successive doses of the blood of tuberculous guinea pigs and by waiting some days after the last injection (as if the phenomenon of Arthus were to be brought about), Helmolz[24] claims to have seen anaphylactic symp-

[20] Compt. rend. Soc. de biol., 1909, **67**, 580.

[21] Wien. klin. Wchnschr., 1908, **21**, 1623;—Compt. rend. Soc. de biol., 1909, **66**, 531; 1910, **68**, 1000.

[22] München. med. Wchnschr., 1909, **56**, 1218.

[23] Ztschr. f. Immunitätsforsch., 1913, **17**, 75.

[24] Ibid., 1909, **3**, 371.

toms produced on the inoculation of a suitable dose of tuberculin. C. R. Austrian,[25] and later F. H. Thiele and Embleton[26] are said to have achieved the same result by introducing into the peritoneum of guinea pigs, previously sensitized by an injection of dead bacilli, the blood of patients who had given the most definite febrile reaction to tuberculin. But animals so sensitized were found incapable of reacting locally to tuberculin.

Lesné and Dreyfus[27] injected serum of tuberculous human cases into guinea pigs into which they afterward inoculated tuberculin by the intracerebral path. Of 100 animals thus treated only 20 reacted. But guinea pigs injected with normal serum gave an identical reaction in 5 per cent of cases when later inoculated intracerebrally with tuberculin. Finally, by using the cerebrospinal fluid of tuberculous individuals for preparatory injection, they succeeded in obtaining a positive reaction in 33 per cent of their animals on injection of tuberculin into the brain. Lesné and Dreyfus came to the conclusion that the results obtained were neither sufficiently constant nor sufficiently specific for one to be able to recommend *anaphylo-diagnosis* of tuberculosis as a clinical measure.

By injecting 2 or 3 cc. of serum from tuberculous patients into the peritoneum of guinea pigs, and then, after 24 to 48 hours, 0.1 cc. of tuberculin, Roepke thought that he observed an elevation of temperature of 1° to 2°, which failed to appear in guinea pigs injected with the serum of normal individuals. But in the opinion of Ernst Fraenkel[28] the rise of temperature here occurring is not at all specific. Sometimes it is lacking following injections of tuberculous sera or exudates, again it is observed when the injection is made with normal serum or indeed with tuberculin alone.

On the other hand, Helmolz,[29] and also Onaka,[30] have found that, if 0.02 cc. to 0.025 cc. of tuberculin is injected intradermically into the skin of the back of guinea pigs which 2 days previously had received an intraperitoneal injection of 4 to 5 cc. of the serum from a man or animal reacting positively to tuberculin, a local reaction is

[25] J. Exper. Med. 1912, **15**, 149.
[26] Ztschr. f. Immunitätsforsch., 1912/13, **16**, 411.
[27] Compt. rend. Soc. de biol., 1909, **66**, 415.
[28] Centralbl. f. Bakt., 1911, **58**, 460.
[29] Ztschr. f. Immunitätsforsch., 1909, **3**, 371.
[30] Ibid., 1910, **5**, 264; **7**, 507.

frequently produced and remains visible for 4 to 5 days in the form of a papular swelling of purplish red color.

But these reactions are certainly not constant, since they could not be reproduced either by Joseph,[31] nor by R. Kraus, E. Löwenstein and R. Volk,[32] C. Vallardi,[33] Neufeld and H. Dold,[34] nor by myself, in spite of numerous attempts.

In my laboratory, L. Bruyant set himself the task of studying this question of the relationship of the tuberculin reaction to the phenomena of anaphylaxis. The first object of his experiments was to establish whether tuberculin really possesses the *anaphylactogenic* property.

Normal guinea pigs received by intracardiac injection doses of 0.005 to 0.01 gm. of dry precipitated tuberculin, diluted with physiological salt solution. Fifteen days later they were tested, along with the controls, with an intracerebral injection of 0.002 gm. of the same tuberculin in 0.2 cc. of physiological salt solution. The percentage of animals which died was the same for the prepared guinea pigs as for those not prepared and did not exceed what is observed following any intracerebral injection whatever. In not one of the prepared animals were any anaphylactic symptoms noted.

A second series of guinea pigs, prepared by the intracerebral injection of 0.002 gm. of tuberculin, gave the same negative results. It seems therefore that tuberculin of itself does not possess any anaphylactogenic property.

Bruyant next sought to determine whether the reaction of tuberculous cases to tuberculin exhibited the characteristics of the anaphylactic reaction. To this end he employed the method of *anti-anaphylaxis,* ingeniously conceived by E. Roux and Besredka.[35]

On the basis of anaphylactic accidents being abolished when the intoxicating injection is made into an animal in a state of narcosis he thought that he would first see whether anesthetics annul the thermic effects of tuberculin injection into tuberculous subjects. But this idea had to be abandoned since anaesthesia with ether, alcohol or chloral regularly causes profound alterations of tempera-

[31] Ztschr. f. Immunitätsforsch., 1909, **4**, 575.

[32] Deutsch. med. Wchnschr., 1911, **37**, 389.

[33] Ztschr. f. Immunitätsforsch., 1910, **7**, 381.

[34] Compt. rend. Soc. de biol., 1911, **70**, 782.

[35] Ann. de l'Inst. Pasteur, 1908, **22**, 496.

ture (marked hypothermia) no matter whether the animals employed are tuberculous or not.

This method failing him, Bruyant turned to the *anti-anaphylactic vaccination* of Besredka, which consists in the injection of a very small quantity of *anaphylactogen* some time before the injection of the intoxicating dose.

Tuberculous guinea pigs were injected intraperitoneally with 0.0001 gm. of dilute tuberculin of *Koch*, a dose already known to be incapable of bringing about an appreciable thermic reaction in these animals.

Three hours afterward, they received 0.002 gm. of Koch's tuberculin subcutaneously, as did at the same time a certain number of equally *tuberculous controls, which however had not undergone the so-called desensitizing injection.* The temperature of all the guinea pigs was taken at the moment of injection and every two hours thereafter.

The rise of temperature, with individual variations, occurred in both vaccinated and non-vaccinated animals, and amounted to 0.5° to 1°C.

In a second series of experiments, tuberculous guinea pigs received intraperitoneally 0.01 gm. of *Koch's* tuberculin. Three hours later they received, along with the controls infected at the same time, a dose of 0.1 gm. intraperitoneally.

The percentage of deaths following the second injection was the same in the vaccinated as in the control animals. In guinea pigs with a great deal of tuberculosis (4 to 6 weeks after infection with 1 centigram of bovine bacilli subcutaneously), the dose of 0.1 gm. of tuberculin introduced into the peritoneum is fatal, with or without a previous vaccinating injection.

J. P. Atkinson and C. R. Fitzpatrick[36] published some analogous experiments carried out upon dogs and which led to the same results.

Instead of employing sera of tuberculous patients or animals to sensitize his dogs, Finzi[37] chose the serum of a horse hyperimmunized with tuberculous endotoxins and human bacilli by the method of Vallée (of Alfort). He thus obtained constantly positive results, in the sense that the guinea pigs prepared with an intraperitoneal

[36] Proc. Soc. Exper. Biol. & Med., 1910, **7**, 77.
[37] Compt. rend. Soc. de biol., 1910, **69**. 4.

injection of serum died in 3 to 5 minutes on being inoculated afterward either intravenously or intracerebrally with very small doses of endotoxin.

Neufeld and Dold[38] repeated these experiments utilizing at times the sera of hypervaccinated goats, of very high agglutinin and precipitin titer, and again antituberculous serum of Ruppel and Rickmann (Hoechst), also strongly agglutinating and giving a complement fixation reaction. They never succeeded in transmitting a passive anaphylactic sensitization to normal animals.

The *lipoids* extracted from the tubercle bacillus are likewise incapable of inducing the phenomena of anaphylaxis or of being utilized to produce anti-anaphylaxis. This fact has been established by the work of Benjamin White.[39] The same applies to the bodies of the bacilli whether killed or living (Delanoe).[40]

E. Friedberger[41] believed that general or local tuberculous reactions could be attributed to the abrupt decomposition of tuberculin or of tuberculous endotoxins by the complement of normal serum in the presence of antibody,—this abrupt cleavage leading to the formation of a new poison (*anaphylatoxin*) to which the characteristic local or general inflammatory phenomena should be due.

The toxic principle, to which Friedberger and his collaborators have given the name of *anaphylatoxin*, is obtained by allowing fresh guinea pig serum to act *in vitro* upon various antigens, sensitized or not. It is a *thermolabile* poison. *Anaphylatoxogenesis* can be realized with a large number of pathogenic micro-organisms, even when killed by heating. It can not be identified with *bacteriolysis*, since the bacterial elements remain intact.

The effects of the *tuberculous anaphylatoxin* are easily studied by varying, against a uniform quantity of antigen (bacilli freshly dried with blotting paper), the proportions of serum and antibody, and the duration of contact.

To prepare the anaphylatoxin, the bacilli are emulsified by triturating in an agate mortar with the immune serum (antibody-containing serum, inactivated by heating at 58°C.). This emulsion is poured into a test tube, left for 48 hours in an ice-box, and then

[38] Arb. a. d. k. Gsndhtsamte, 1912, **38**, 278.
[39] J. Med. Research, 1914, **30**, 393.
[40] J. de physiol. et de path. gén., 1909, **11**, 441.
[41] Ztschr. f. Immunitätsforsch., 1911, **9**, 369; 431.

centrifugated. The bacilli are washed in physiological salt solution and then suspended in a quantity of complement corresponding to 4 cc. of fresh guinea pig serum for each 0.3 gm. of bacilli. This mixture is placed in the incubator or water bath for 1 to 2 hours at 37°C., and then in an ice box for 20 hours. After centrifugating, the supernatant fluid is decanted and injected in a dose of 3 to 4 cc. into the jugular vein of young *normal* guinea pigs whose weight should not exceed 200 grams.

The animals thus inoculated die in 2 to 3 minutes with symptoms simulating those of anaphylactic shock.

One may treat the same bacilli several successive times with a new dose of complement and obtain in each instance a new quantity of anaphylatoxin, but the yield is less and less and finally not enough is obtained to kill.

Repetition of the preliminary sensitization with immune serum is not indispensable. According to Friedberger's[42] experiments, it does not even seem that this sensitization is necessary. It may be omitted. The *anaphylatoxogenesis* is accomplished through the simple action of antigen on guinea pig complement.

G. Shibayama observed that bacilli freed of fat without heat by means of ether or alcohol can form anaphylatoxin more regularly than bacilli direct from the cultures and possessed of their waxy fatty components.

Finally, from investigations of H. Dold and Hanau,[43] bacilli *freed of anaphylatoxin* by normal guinea pig serum are said to be no longer capable of producing anaphylatoxin when injected *in vivo* into the peritoneum, although they are themselves still toxic. One should therefore distinguish the *anaphylatoxic* action from the *endotoxic* action, properly speaking, of the bacilli.

The experiments of Wassermann and Keysser,[44] Ritz and Sachs,[45] of Bauer,[46] Doerr and Pick,[47] of S. Mutermilch,[48] and above all those

[42] Ztschr. f. Immunitätsforsch., 1913, **18**, 344.
[43] Ibid., 1913, **19**, 31.
[44] Folia Serol., 1911, 7.
[45] Berl. klin. Wchnschr., 1911, **48**, 987.
[46] Ibid., 1912, **49**, 344.
[47] Wien. klin. Wchnschr., 1912, **25**, 331.
[48] Ann. de l'Inst. Pasteur, 1913, **27**, 83.

of A. Besredka, H. Ströbel and F. Jupille,[49] make it possible to explain *anaphylatoxogenesis* otherwise.

The fact that one may obtain anaphylatoxin by treating fresh guinea pig serum with various inorganic substances (*kaolin, barium sulphate*), with agar or pure peptone, indicates that *anaphylatoxic action* should not be regarded as produced by a toxin, but rather is the manifestation of a phenomenon resulting from an alteration in the physical state of normal serum,—an alteration induced by *absorption* of certain protective or antagonistic substances normally present in fresh serum. Among these are *complement* and perhaps also *lipoids* (non-saturated fatty acids), as some recently published and very interesting investigations by James W. Jobling and William Petersen[50] would indicate.

The ultimate conclusion to be drawn from all the foregoing facts appears then to be that *the tuberculin reaction in the tuberculous cannot be regarded either as a phenomenon of anaphylaxis or as an anaphylatoxin reaction.* It results from a *lytic action* on the part of certain substances, contained in the body fluids of subjects *infected with the bacilli,* upon the tuberculin; and *this lytic action gives rise to the formation of a specific product, both toxic and fever producing,* which is the essential factor in general or local tuberculin reactions. *When this lysis is too intense and severe it may cause death through an over-acute intoxication.*

D. ACTION OF TUBERCULINS UPON THE CELLULAR ELEMENTS

If one studies histologically the changes brought about in the tissues, such as the skin or mucous membranes, by the inoculation or instillation of a small quantity of tuberculin in a tuberculous subject, it is found that phenomena of hyperemia are produced at the point of inoculation or instillation after a very brief delay. These phenomena are characterized by an accumulation of polynuclear leucocytes, followed in turn by a lymphocytic mononucleosis accompanying a moderate exudation of lymph which remains localized beneath the ordinarily intact superficial epithelium. At the same time there is observed a somewhat marked capillary vaso-dilatation. There is thus formed—depending upon the point

[49] Ann. de l'Inst. Pasteur, 1913, **27**, 185.
[50] J. Exper. Med., 1914, **20**, 37.

of application of the tuberculin, the degree of tuberculous infection and the quantity of tuberculin inoculated or instilled—a local lesion of varying intensity, assuming the aspect of a small acute subcutaneous lymphoma, or that of a papule surrounded by an inflammatory zone (cuti-, intradermo-, ophthalmo-reactions).

The experiments of Von Lingelsheim and those of A. Borrel had shown that certain fixed cellular elements, such as the nervous cells, possess also a very great affinity for tuberculin. G. Guillain and G. Laroche[51] observed that its fixation could even be accomplished *in vitro*. On leaving in contact, in an ice box, for 16 to 24 hours, an emulsion of human or guinea pig brain mixed with tuberculin, and by afterward eliminating the excess tuberculin by successive washings and centrifugations, it is found that the tuberculin treated brain tissue is toxic for tuberculous guinea pigs when injected intracerebrally in a dose of 0.2 cc. An equal amount of an emulsion of normal brain, on the contrary, causes no symptoms. Guillain and Laroche believe even that tuberculin acquires a greater toxicity, weight for weight, from the fact of its fixation by nervous tissue. It is probable therefore that, in tuberculous subjects, the ferments capable of producing lysis of tuberculin and of thus creating the specifically active tuberculous toxin, are elaborated, not only by the leucocytes, but also by certain fixed cellular elements, such as the nerve cells.

It should be recalled that, in tuberculous man and animals, attention has been drawn to the diminution in the number of leucocytes in the blood following upon injections of tuberculin, regardless of whether the latter are made for diagnostic or therapeutic purposes (*see Chapter XXXII*).

E. RESISTANCE AND TOLERANCE OF THE TUBERCULOUS ORGANISM TO TUBERCULIN

As early as 1892 Nocard had called the attention of veterinarians to the fact observed in tuberculous cattle that reactions following injections of tuberculin repeated at intervals of a few days, became less and less intense. Moreover he advised waiting about one month before making a new diagnostic injection into suspected animals. Vallée[52] (of Alfort) later suggested a very simple means of avoiding

[51] Compt. rend. Soc. de biol., 1910, **68**, 220.
[52] Ann. de l'Inst. Pasteur, 1904, **18**, 545.

this difficulty: namely, to inject a dose of tuberculin twice as great as the one ordinarily used (for example, 8 cc. of 1 in 10 tuberculin for large animals, instead of 4 cc.) (*see Chapter XXIV*).

Among tuberculous patients treated with the different tuberculins in sanatoria, this tolerance has long been noticed; by virtue of it patients can be gradually made to tolerate very large doses of the substance, provided the injections are properly spaced and do not produce febrile reactions. Upon the basis of this action the whole present day tuberculin therapy of tuberculosis is built up.

Experimentally this tolerance is found to be limitless, so to speak. Thus Et. Burnet,[53] taking guinea pigs on the thirtieth day after they had been infected with tuberculosis, and beginning with the subcutaneous injection of 1 mgm. of tuberculin (precipitated) every day, was able to carry his animals on to 100 mgms. on about the seventieth day of their disease. Some of the animals had absorbed 360 mgms. of precipitated tuberculin during 40 days.

Now these guinea pigs, although they had become very resistant always gave a thermal reaction to massive doses; although the reaction was a little retarded. Their serum moreover contained nothing capable of neutralizing tuberculin *in vitro* nor of passively sensitizing normal guinea pigs against this poison.

A. Manaud,[54] in my laboratory, obtained the same results. He found too that tuberculous guinea pigs, worked up to enormous doses of tuberculin (as high as 200 mgms. of precipitated product), lose their resistance fairly rapidly as soon as the injections are stopped. Thus after 26 days of rest, certain animals succumbed to a dose of 50 mgms., whereas others, of the same series, which had continued to receive 200 mgms. every 2 or 3 days, tolerated it perfectly.

A. Manaud found, on the other hand, that in guinea pigs thus inured to resist tuberculin, the tuberculous lesions continue to progress as in the controls, or even a little faster. *There does not seem therefore to be any correlation*—at least in the guinea pig—*between resistance to tuberculin* and *resistance to tuberculous infection*

[53] Compt. rend. Soc. de biol., 1908, **65**, 307.
[54] Ibid., 1909, **66**, 502.

F. INFLUENCE OF TUBERCULIN UPON THE MOBILIZATION OF BACILLI IN THE ORGANISM

The often harmful effect of diagnostic subcutaneous injections of tuberculin upon the evolution of tuberculosis in man was remarked by physicians and led to the assumption that the general febrile reaction provokes the mobilization of bacilli, until then sequestered in nodular lesions, with a *bacillemia*. Some observers, among whom R. Virchow and Orth as early as 1891, then Liebmann and L. Rabinowitsch,[55] Israel Bacmeister,[56] Moores and Brautigam, and Hans Kohn, upheld this idea, while others opposed it (Arima and Tanaka).

I had this question restudied in my laboratory by L. Massol and M. Breton,[57] using the method of transfusion, the technique of which was described in a preceding chapter (XVIII).

A first series of guinea pigs received uniformly 1 mgm. of a culture of bovine bacilli (weighed fresh), under the skin of the thigh. The series was then divided into four equal lots. The first, composed of 8 guinea pigs, was kept as controls. The second was injected with 0.05 cc. of *Koch's* old tuberculin on the same day as the bovine bacilli. The third and fourth lots received the same dose of tuberculin, respectively 2 days and 1 day before the transfusion. The transfusion was performed on the tenth day after virulent infection, by reason of the fact that, under such experimental conditions, bacillemia acquires its maximum intensity at this time.

When the recipient animals were killed and autopsied 45 days after transfusion their lesions were found equally advanced in all cases. Consequently one cannot say that after tuberculization, even with a massive dose (1 mgm.), tuberculin plays a fixative rôle as regards bacilli circulating in the blood.

Furthermore it could be proved, always in the guinea pig, that a tuberculin treatment beginning with a dose of 0.01 cc. (*Koch's* old tuberculin) and increasing progressively up to 1 cc. on the twentieth day, is also incapable of preventing bacillemia.

A second series of guinea pigs was infected with minimal doses of bacilli (0.01, 0.001, 0.0001, 0.00001 mgm.) in order to reduce the bacillemia to a minimum and in order to note its variation in degree under the influence of tuberculin.

[55] Berl. klin. Wchnschr., 1913, **50**, 110.

[56] München. med. Wchnschr., 1913, **60**, 343.

[57] Compt. rend. Soc. de biol., 1914, **77**, 362

This series was divided into 4 lots of 18 guinea pigs each, and subdivided into two categories, one of control animals and the other of those injected with tuberculin. Tuberculin was administered in increasing doses during the 7 days preceding transfusion, which took place 41 days after infection. The quantity of tuberculin varied between 0.01 and 0.1 cc.

The animals injected with tuberculin and *killed 5 months after being infected*, were, from the pathological point of view, comparable with the control guinea pigs. Each of them in like manner presented very small glandular and splenic lesions, compatible with a long survival. *The tuberculin did not favor the bacillemia.* The progress of the tuberculosis in the blood donors was not hastened as compared with the controls.

Under the above-specified experimental conditions, it is therefore evident that tuberculin neither induces nor inhibits bacillèmia. It plays no rôle in the dissemination of the bacilli in the blood and it seems that Koch was correct in his early opinion against mobilization of bacilli by tuberculin.

CHAPTER XXXVI

DIAGNOSIS OF TUBERCULOUS INFECTION BY TUBERCULIN REACTIONS

As soon as Robert Koch in 1890 made known the curious properties of his "lymph" it was applied to the treatment of patients. Not until later was the true import of this great discovery to be determined. It was recognized, particularly after investigations undertaken by Guttmann (of Dorpat), by Roeckl and Schutz, Lydtin in Germany, by Bang and Salomonsen in Denmark and by Nocard in France, that tuberculin was to furnish veterinary medicine with a marvellous means of detecting tuberculosis in cattle, when neither physical examination nor the presence of bacilli in expectoration or in milk could give the information. Then the clinicians, who were experimenting with tuberculin upon a large scale from a therapeutic point of view and meeting with frequent and cruel disappointments, came to realize that this valuable substance was to be used indeed more advantageously for the diagnosis of incipient or doubtful cases of tuberculous infection.

A. GENERAL OR SUBCUTANEOUS TUBERCULIN REACTION

The first method to be adopted was that of subcutaneous injection, which, in subjects who have bacillary lesions no matter how slight, constantly produces a general reaction manifested by a rise of temperature. The reaction varies considerably with the dose of tuberculin employed and the age of the lesions. As a general rule, the less extensive the lesions the more intense is the reaction. A case of glandular tuberculosis presenting only suspicious clinical signs for example, if injected with 0.001 gm. of *Koch's* old tuberculin, begins to show an evident temperature reaction after about 4 hours. The temperature rises progressively to near 39.5°, at times to 40° at about the twelfth hour, then falls rather rapidly, to return to normal at approximately the twentieth hour.

It was soon recognized that large doses of tuberculin may have serious effects upon patients because of the focal reactions which

always accompany and persist longer than the temperature reaction. These focal reactions all too frequently whip up the disease and hasten its progress. Large doses therefore had to be given up and all clinicians now agree that tuberculin as a diagnostic measure should not be used in doses exceeding 0.0001 gm. (0.1 mgm.) in adults and 0.00005 gm. (0.05 mgm.) in children.

Even with such doses tuberculin diagnosis by subcutaneous injection is contraindicated under many circumstances. It should not be used in persons who already have irregularities of temperature, and still less in those with fever. It must also be carefully avoided in persons with recent or old hemoptyses, in convalescents from infectious diseases, heart cases, albuminurics, diabetics, and patients with affections of the nervous system or sense organs.

In addition to the possibly serious focal reactions, there are objections to the subcutaneous injection which make many physicians hesitate before using it. The fever set up is accompanied at times by very sharp headache, lumbago and severe vomiting. Consequently it is scarcely used at all nowadays except in surgical or dermatological services, particularly in children, in cases where other diagnostic measures have given uncertain results and where there is still reason for suspecting or ruling out the diagnosis of tuberculous infection.

In these exceptional circumstances, the technique most to be recommended is the following:

The patient's temperature should be taken by mouth every three hours during the two days immediately before the test. Subjects having maximum temperatures above 37.3° should not be injected, or else they should be kept in bed until their temperature has returned to normal.

It is best to inject the tuberculin very early in the morning. If done in the evening, the mild reactions, coming on in barely 6 hours, will pass unnoticed during sleep and will have completely disappeared by morning. Then too, certain delayed reactions, which begin occasionally after 30 hours and are more or less transitory, would likewise escape observation.

The temperature should be taken punctually every two hours (every three hours at night) until the end of the second day.

The solution to be injected is best prepared just before use. To a flask containing 100 cc. of previously sterilized 0.5 per cent carbolic

water or simple physiological salt solution are added 2 drops of *Koch's* tuberculin raw. The flask is corked, shaken a few moments to assure thorough mixing, and the fluid drawn up directly in a sterile syringe. One cubic centimeter of this solution contains 1 milligram of raw tuberculin. Therefore one can inject 0.1 cc. which corresponds to 0.1 mgm. or, if necessary, make higher dilutions for injecting smaller doses.

The injection is made aseptically in the subcutaneous cellular tissue, preferably of the antero-external surface of the thigh.

It is generally agreed that the reaction is positive when the maximum elevation of temperature has been at least 0.7°C.

There often occurs at the site of injection a painless reddening which persists for several days. It is caused by a small quantity of tuberculin penetrating into the dermis and is then equivalent, as regards diagnostic significance, to the *intradermic* reaction of which we shall speak a little later.

When a first injection of tuberculin in very small amount has given negative results, the indication may be to repeat the test after 8 to 10 days with a somewhat larger dose, or else as recommended by Moeller, Lowenstein and Ostrowski,[1] to reinject the initial dose every three or four days until a reaction ensues. Thus are obtained the effects of an accumulation which do not present the dangers of a single large dose. Yet this test should never be repeated more than five successive times, since accumulation may end by intoxicating even a normal person and by provoking a mild febrile reaction (Paul Claisse,[2] Slatineanu, Danielopolu and Ciuca[3]). Certain individuals have been noted who failed to react to several large injections of tuberculin (0.01 gm.) and then reacted to a single injection of the same dose 15 days later.

B. TUBERCULIN REACTION BY RECTAL ABSORPTION

A tuberculin test may have to be performed without the patient's knowledge or in a manner to avoid any painful or impressive effect such as may arise from subcutaneous injection. Under such circumstances it will be found well to try the introduction of tuberculin

[1] Internat. Congr. on Tuberculosis, Paris, 1905.

[2] Bull. et mém. Soc. méd. d. hôp. de Par., 1907, 3. s., **24**, 689.

[3] Compt. rend. Soc. de biol., 1910, **68**, 903.

by the rectum. It must be understood however that this procedure is now and then unreliable, except in young children, since absorption from the large intestine varies greatly in different individuals.

I have demonstrated by experiments in collaboration with M. Breton and J. Minet[4] that an enema containing 0.01 gm. of tuberculin mixed in 50 cc. of milk, produces the characteristic febrile reaction almost constantly. The objections and contraindications to this method are the same as with subcutaneous injection.

C. CUTI-REACTION OF VON PIRQUET

As early as 1903 von Pirquet[5] had advanced the hypothesis that the reaction of tuberculous cases to tuberculin, like that of subjects sensitized to serum, and like the premature cowpox reactions or vaccinella, was due to a phenomenon of sensitization to which he gave the name of *allergy*. This was the point of departure for a series of studies which led him in 1907 to the discovery of the cutaneous (or cuti-) tuberculin reaction (*Plate XXV*).

This reaction consists in introducing a small drop of tuberculin into the skin through a simple scarification or pricking. If the subject is tuberculous there appears after 10 to 24 hours, occasionally a little later, a very characteristic papule of purplish red color. This papule remains visible for several days and then disappears without leaving any trace. In subjects free from tuberculous lesions, the same inoculation remains almost constantly without effect. It very much resembles certain *tuberculides* encountered rather often upon the skin of the face or other exposed parts of the body, particularly in children (*Plate XXV, 4*).

The technique of the test is very simple, in spite of all the variations proposed by different authors.

The following appears the most to be recommended:

Having satisfied oneself that the skin area to be tested (for example the antero-external surface of the arm, as though vaccinating against smallpox) presents no alterations, it is cleaned with a sterile cotton pledget moistened with alcohol, ether or sterile water, and then wiped dry with another pledget.

With a flamed vaccine needle, three scarifications are made, 3 to 4 mm. long and at intervals of a few centimeters. They should

[4] Compt. rend. Soc. de biol., 1908, **64**, 163.

[5] Deutsch. med. Wchnschr., 1907, **33**, 865; 905.

PLATE XXV

1. Tuberculin cuti-reactions with gradually increasing doses of tuberculin (from above downward: dilution $\frac{1}{4}$, $\frac{1}{10}$, $\frac{1}{20}$, $\frac{1}{50}$).

2. Positive ophthalmo-reaction, of moderate intensity, in the right eye (left of the figure).

3. Positive intradermic reaction (from Ch. Mantoux), on a child's thigh.

4. Tuberculous lupus of the cheek, in a child (from a model at the Hôpital St. Louis).


I
II
III
IV

involve the dermis but slightly and not cause bleeding. Upon two of these scarifications one places and spreads a drop of raw tuberculin of Koch diluted 1 in 4 in sterilized glycerin. The third scarification serves as a control and receives no tuberculin.

The scarified region is left open to the air for a few minutes, then covered with a light cotton dressing which may be removed after 2 to 3 hours.

The individual is afterward examined after 24 and after 48 hours.

If the reaction is negative, the scarifications impregnated with tuberculin behave just as does the control. They heal very rapidly under a small brownish crust, without any induration or diffuse reddening.

If the reaction is to be positive, a slight dermo-epidermal swelling appears by the tenth hour and is much more accentuated by the 24th hour. It is at first of a pink color and later dark red. There is some edema at the margin of this papule, and rather more color in the center, which is slightly raised.

The reaction is like a papulo-erythematous patch. Its margins may be either regular or irregular. Its red tint becomes deeper and often of a purplish hue.

The intensity varies. The diameter is from 4 or 5 mm. to 2 cm. or more and, at the periphery, tiny hemorrhagic points or little vesicles filled with clear fluid are seen to appear. However it never becomes vesicular in the sense of a vaccination pustule. The little blebs and the papule disappear in some 4 to 8 days and are replaced by a delicate crust which breaks and falls off without leaving a scar.

Certain mild reactions, which are observed chiefly in extremely tuberculous subjects, do not appear except in the form of a small erythematous area a few millimeters in size. The latter usually suffices however to indicate its specificity.

The characteristic feature of a positive reaction is the induration of the papule to the sense of touch. When grasped between the fingers it gives a very distinct sensation of being elastic.

In young children and particularly in nursing infants the redness rarely persists beyond the third day.

V. Tedeschi[6] proposed, at one time, to make the inoculation into the auricle of the ear, on the ground that the substratum of cartilage

[6] Pediatria, 1909, 2. s., 7, 641.

in this region would make the induration more evident. This idea does not seem to be altogether a happy one, since there would be more risk of contamination of the small wound and resulting swelling of the retro-auricular glands.

The cuti-reaction when positive causes a very moderate itching, but none of the bad effects of the subcutaneous injection of tuberculin. Fever is never observed, nor any influence upon near or distant tuberculous foci. Contraindications are therefore exceptionally few. Nevertheless it should not be employed in subjects with tuberculous lesions of the skin, nor in those affected with cutaneous troubles such as impetigo, ecthyma, eczema, etc., nor in children in the course of the exanthemata.

Sezary[7] has quite rightly insisted upon the fact, already pointed out also by Gavet,[8] by Léon Bernard and Baron,[9] that in the course of an acute, severe or intense infection, the cuti-reaction is frequently negative, although found positive before the acute disease and noted as reappearing during convalescence.

This fact has been demonstrated chiefly in measles (von Pirquet, Moltchanoff) and in typoid fever, where it has been observed in about one-third of the cases, in pneumonia more often still, in bronchopneumonia, in rheumatic fever, scarlatina, diphtheria, erysipelas, malaria, etc. It is said to have been noted even during pregnancy (Gavet).

On microscopic examination of a section of the cuti-reaction papule, excised perpendicularly to the skin and from a living subject, there is found according to Pfandler:[10]

1. An apparently unmodified epidermis beneath which the papillary and reticular layers of the dermis, as well as the more superficial levels of the subcutaneous tissue, are found infiltrated with collections of leucocytic elements.

The latter become less dense away from the line of scarification. They have their seat at the base of the papillae, about the hair follicles, sweat glands and capillaries. They are everywhere *perivascular* and joined together by strands.

[7] Gaz. des hôp., 1913, **86**, 1789.

[8] Thèse, Paris, 1912.

[9] Presse méd., 1912, i, 505.

[10] München. med. Wchnschr., 1907, **54**, 1417; 1482; 1532.

2. With a considerably higher magnification it is seen that the tissues contain a large number of mononuclears which have taken the place of the polynuclears still visible in the crust, also some of the *germinative cells* of Flemming, recognized by their large size, their basophile protoplasm, and their large clear nuclei containing coarse granules of chromatin.

The papillae, in the neighborhood of the scarification, are distended by a considerable edema which separates or forces back the connective tissue. The edema contains polymorphonuclear cells clumped about the blood vessels.

This inflammatory process is characterized therefore by a very distinct mononucleosis and by the presence of numerous germinative cells of Flemming.

Certain authors (Pick and Daels,[11] Zeiler[12]), by using tuberculins containing the bodies of the bacilli, have observed in cuti-reactions the formation of giant cells surrounded by a certain number of epithelioid cells.

Bandler and Kreibich, Ferraud and Lemaire,[13] have also called attention to analogous formations, which however, in their opinion, have not the characters of the true tuberculous giant cells of Langhans.

D. MODIFICATIONS OF THE CUTI-REACTION

a. Procedure of Lignières[14]

This consists in shaving the skin and quickly rubbing in a few drops of raw tuberculin. In tuberculous cases papules appear whose color varies from pink to deep red. They are generally surrounded by an areola and often collect to form little confluent islands or an edematous patch. The papules disappear after 4 or 5 days or are transformed into vesico-pustules with crust formation.

The patient notes an itching sensation but has no fever nor general symptoms.

[11] Med. klin., 1908, **4**, 58.
[12] München. med. Wchnschr., 1908, **55**, 1685.
[13] Presse méd., 1907, ii, 617.
[14] Centralbl. f. Bakt., 1908, **46**, 373.

b. Procedure of Lautier[15]

Without any preparation of the skin, a small rather loose pledget of absorbent cotton moistened with 2 or 3 drops of 1 per cent tuberculin is applied to the external surface of the arm. The pledget having been covered with rubber tissue to prolong the contact of the tuberculin with the skin, the whole is surrounded with cotton and a bandage and left in place for 48 hours.

After the dressing has been removed to note the result, it is necessary to wait one or two hours for the reaction to assert itself. When positive, it consists either in a single papular erythematous patch or of numerous small isolated areas. Its color is pale pink or slightly copper colored. The skin, thick and swollen, has a dry wrinkled feeling. The surface under a magnifying glass is studded with very fine small vesicles containing a colorless fluid. This eruption continues from 2 to 20 days, with itching.

c. Transcutaneous reaction of Moro[16]

This consists in rubbing into the skin a pomade composed of equal parts of the raw tuberculin of Koch and of lanolin. Care is taken to gently warm the lanolin before incorporating the tuberculin, in order that the mixture shall be homogeneous. The pomade can be preserved over a long period in a tight jar and in a cool place.

The rubbing should be performed over an area 5 cm. in diameter, preferably in the epigastric region or in the neighborhood of the nipples. It should be continued from 30 to 60 seconds. The part rubbed is afterward left exposed to the air for about ten minutes. A covering dressing is unnecessary.

When the reaction is negative, the skin remains absolutely normal. In the case of a positive reaction, many small red papules are formed after 24 hours, to persist for several days. There is generally a sensation of slight itching at the beginning of the reaction.

d. Rhino-reaction

Laffitte-Dupont and Molinier[17] and later Möller,[18] proposed the application of a 1 per cent solution of tuberculin to the nasal mucosa,

[15] Compt. rend. Soc. de biol., 1908, **64**, 91.
[16] München. med. Wchnschr., 1908, **55**, 217.
[17] Compt. rend. Soc. de biol., 1908, **64**, 702.
[18] München. med. Wchnschr., 1908, **55**, 2324.

either by spreading a drop upon the surface of the inferior turbinate, or by placing for 10 minutes, upon the mucous membrane of the septum, a small tampon of absorbant cotton moistened with the same solution. A positive reaction is characterized by a small thin transparent crust which forms from the second to the fourth day upon the congested mucosa.

e. Urethral and vaginal reactions

On the same principle, Oppenheim[19] instilled tuberculin into the urethra. But the reaction thus produced is weak and inconstant. Schwab[20] has done the same with the vagina with no better results.

f. Subcutaneous local reaction (Stichreaktion) of Escherich[21] and Hamburger

F. Hamburger[22] injects with a syringe one-tenth of a milligram of Koch's old tuberculin into the dermis of the external aspect of the forearm, avoiding the introduction of the needle into the subcutaneous cellular tissue, just as in the intradermic reaction of Ch. Mantoux, which will be discussed a little further on. Twenty-four hours after the injection two inflammatory zones are observed, of which one develops about the point of inoculation and the other at the point where the needle pierced the epidermis. The redness and swelling increase in intensity for 24 to 48 hours, to disappear a few days afterward. A brownish colored thickening of the skin persists a fairly long time.

This reaction is painful; it leads to an edematous infiltration and a fairly intense pruritis; at times there is some fever and, particularly when the test is performed upon the arm, an engorgement of the corresponding glands (Rist).[23] Therefore the "stichreaktion" of Escherich presents the same contraindications and some of the same objectionable features as the subcutaneous reaction.

[19] Wien. klin. Wchnschr., 1908, **21**, 1294.
[20] München. med. Wchnschr., 1908, **55**, 1609.
[21] Jahr. f. Kinderheilk., 1892, **33**, 369.
[22] Wien. klin. Wchnschr., 1908, **21**, 381.
[23] Bull. Soc. d'étude scient. sur la tuberc., 1913, Feb.

E. INTRADERMAL REACTION OF CH. MANTOUX

This procedure was proposed as a clinical measure by Ch. Mantoux[24] and was introduced by Moussu into veterinary medicine. It consists in injecting a *measured* quantity of tuberculin *into the dermis.* The technique according to Mantoux is as follows:

"Nothing in the way of an instrument is required except an ordinary hypodermic syringe with graduated screw control piston, and a fine needle. A 1 in 5000 solution is prepared by diluting a one cubic centimeter ampule of stock 1 per cent tuberculin from the Pasteur Institute with 49 cc. of physiological salt solution. Of this dilution one drop, that is 0.01 mgm., is injected into the anterior surface of the thigh. The skin is pinched up and the needle introduced almost parallel to the surface, care being taken that the bevelled side be turned outward and consequently toward the epidermis, not toward the hypodermis, when the needle is in place. In subjects with very delicate skin the needle should be boldly inserted into the subcutaneous tissue and then slightly tilted so as to reach the dermis from beneath; otherwise there is the risk of going right through.

"Except for this little trick, the procedure is exactly like a tracing injection of cocaine; the needle being well fixed, the liquid is introduced and forms a small wheal of edema which is quickly reabsorbed."

One may add to the solution of tuberculin 1/200 of stovain hydrochlorate as indicated by Mantoux, thus making the injection less painful.

It is always preferable to prepare the 1 to 5000 solutions of tuberculin just before using, since very high dilutions quickly lose their activity.

The syringe used should not leak and the piston should be accurately adjusted to the barrel in order that the fluid, in meeting with a very stout resistance in penetrating into the dermis, shall not be able to flow back around the piston. Furthermore a short, but strong and fine needle should be chosen.

When the reaction is to be positive, it is already visible within a few hours. At 48 hours it reaches its maximum. There is a central nodular infiltration, pink or bright red, surrounded by a halo of

[24] Compt. rend. Acad. des sci., 1908, **147**, 355;—Compt. rend. Soc. de biol., 1909, **67**, 54; 436; 665.

pink erythema. The infiltration at the center may be 1 to 3 cm. in diameter; the peripheral halo varies greatly in extent and may be even as broad as the surface of the palm of the hand (*Plate XXV, 3*).

The skin is hot and rather sensitive, and gives a sensation of thickening of the dermis. In certain patients, according to Chauffard and Jean Troisier,[25] the reaction reminds one of a rather pink urticaria papule, or again of a small patch of erythema nodosum.

In some cases, which are rare however, the reaction is delayed, not appearing until the third to the fifth day.

As a general rule, it begins to recede after 48 hours. The halo disappears rapidly, but the central nodule persists for several days. A pigmented trace can still be seen after several weeks.

When the reaction is negative, the slight needle traumatism is practically invisible after 48 hours.

In patients with non-tuberculous dermatoses, one often obtains reactions which reproduce the type of skin lesions with which the individual is affected. Such reactions are not to be regarded as positive. They appear and disappear more quickly than in the tuberculous.

In laboratory animals, the guinea pig in particular, Ch. Mantoux recommends performing the intradermic test by inoculating one drop of a standard 1 per cent solution of precipitated tuberculin from the Pasteur Institute. He chooses the external surface of the hind paws which are previously depilated. The skin is held tight against the subjacent osteo-muscular layer by stretching it between the two fingers and the injection is given as in man.

A positive reaction consists in a white or pink edematous infiltration of the dermis, accompanied often by a hemorrhagic suffusion. It attains its complete development with a diameter of from 12 to 18 millimeters 48 hours after inoculation.

When the reaction is negative, all trace has disappeared by the end of the second day.

Ch. Mantoux has noted that artificially infected guinea pigs do not react for a rather variable time after the infecting inoculation,—I should add fairly late,—which indicates that the reaction is not very sensitive, at least for laboratory animals.

However, J. Blanco[26] says that he has obtained excellent results and

[25] Bull. et mém. Soc. méd. d. hôp. de Par., 1909, 3. s., **26**, 7.
[26] Bol. de Inst. nac. de Hig. de Alfonso XIII, 1917, March 31.

early responses by depilating a small non-pigmented portion of the skin of the lumbar region or flanks of guinea pigs with a mixture of equal parts of barium sulphide and calcium carbonate. He then injects into the dermis, within 24 hours, 0.1 cc. of a 1 to 5 dilution of Koch's old tuberculin in physiological saline (that is 0.02 cc. of raw tuberculin). In order that the reaction may be regarded as specific, the infiltration should last for 48 hours and the redness should be very apparent within 24 hours. Reactions both weak and strong are said to have been observed from the seventh to the fifteenth day after infection.

Ch. Mantoux and Perroy[27] determined the reactivity of non-tuberculous pigs previously injected subcutaneously with tuberculin (0.2 to 0.5 cc. of raw tuberculin) to intradermal injection. From their experiments, there is no doubt that such a reaction occurs, but it is always less definite in guinea pigs treated with tuberculin than in tuberculous pigs, and it does not appear until between the 10th and 39th days.

B. Auché and Augistron,[28] later Paul Spehl[29] studied the histology of the intradermal reaction as obtained in the tuberculous guinea pig. They found that the superficial layers of the epidermis are filled with leucocytic nuclei and their fragments. The middle portion of the stratum mucosum of Malpighi is broken up over a large area by a band of cellular infiltration made up exclusively of well preserved polynuclear leucocytes. In addition many epidermal cells were found to have undergone vacuolar changes.

The infiltration of polynuclears, which increases up to the end of the second day, invades also the dermis and the subcutaneous cellular tissue as far as the first subjacent striated muscular fibres. The blood capillaries are dilated and the connective tissue infiltrated with red cells.

F. OPHTHALMIC REACTION OF WOLFF-EISNER-CALMETTE

Simultaneously and independently of one another, Wolff-Eisner and A. Calmette[30] studied and described characteristic reactions which were obtained by simply instilling one drop of dilute tuberculin

[27] Compt. rend. Soc. de biol., 1911, **70**, 974.
[28] Ibid., 1910, **68**, 330.
[29] Arch. méd. expér., 1913, **25**, 239.
[30] Compt. rend. Acad. des sci., 1907, **144**, 1324.

upon the ocular conjunctiva of tuberculous human beings or animals. The technique which we have recommended is very simple, and it was quickly introduced into clinical practice. During the 2 or 3 years which followed the publication of our first investigations, an immense number of observations were gathered almost everywhere, often bringing out a comparison of the different local tuberculin reactions. The result is that we are today well informed as to their diagnostic value, as well as to their respective advantages and disadvantages.

The tuberculin is instilled with a dropper or fine pipette at the inner canthus of one eye, a single drop of a sterile 1 per cent solution of precipitated purified tuberculin being used. The head should be tilted back. With the left hand the lids are held apart, the patient being instructed to look upward and outward; with the right hand the drop is instilled, another one being immediately introduced if the first is forced out by a spasm of the lids. The eye is covered with a pledget of cotton soaked in boiled water and with a band. These are removed after a few minutes.

When the reaction is to be positive there is seen after 5 to 6 hours a little reddening of the conjunctiva; little by little this increases in extent and in 24 to 48 hours generally reaches its maximum intensity (*Plate XXV, 2*). The instillation ought therefore to be made in the morning, so that the results may be observed the same evening, the next morning, the evening of the next day and the second day. Delayed reactions are rare. When reactions occur with their normal intensity, the caruncle, the semi-lunar fold, and the conjunctiva are found red and swollen, and some exudate, at first serous and then slightly cloudy, accumulates in the conjunctival sac. The eye lachrymates and the subject complains of a painful pricking sensation, altogether like that experienced when a small foreign body irritates the conjunctiva. The sub-conjunctival capillaries are dilated and small ecchymoses are now and then produced.

After 2 or 3 days the local inflammation subsides and disappears, but frequently the eye remains red for one or two weeks. These prolonged reactions are observed particularly among moderately infected or very resistant individuals.

Subjects who are entirely free from all tuberculous infection do not react in any way to the tuberculin instillation. Yet one occasionally observes a mild reddening, after 2 or 3 hours, if glycerinated tuber-

culin or badly sterilized solutions have been employed. But this redness disappears very quickly, is not accompanied by lachrymation, and the caruncle does not take on the deep red color of the positive reaction.

Cytological study of the conjunctival secretion induced by tuberculin in tuberculous individuals shows a marked predominance of polynuclear leucocytes and degenerated epithelioid cells (Sabrazès[31] Mongour and Brandeis,[32] Lafon and Lautier,[33] G. Stanculeanu and Mihail.[34] With biopsy the conjunctiva is found infiltrated with large mononuclears almost all of which show a more or less extensive vacuole, an indication of secretory hyperactivity. The blood capillaries contain numerous lymphocytes and some basophile polynuclears (Mastzellen) which emigrate into the perivascular connective tissue.

The instillation of tuberculin is a particularly convenient procedure in the young, and we shall see later that its use should be restricted almost exclusively to diagnosis of tuberculous infection in early childhood. Beyond the age of 5 years it is no longer to be recommended.

In any case, before performing it, one must be certain that the eyes are normal, since distressing accidents have been reported as occurring in some individuals where the conjunctival inflammation was so extensive as to set up a keratitis and even ulceration of the cornea. It should be remarked that such complications are not in reality to be attributed to the instillation of tuberculin but solely to the lack of cleanliness and care. If the tuberculin used is properly prepared and sterile, it is incapable of producing such disturbances.

This valuable method of diagnosis has been criticized more justly because of another disadvantage which cannot be obviated, namely, that it informs very exactly not only the physician but the patient himself and the others about him as to the result of the test. Consequently, after having used it on a perhaps exaggerated scale, clinicians have almost abandoned it, and unjustly to my mind, since it is capable of greatly aiding in prophylactic measures against tuberculosis, *by detecting contagion within the family at an early period.*

[31] Folia Hematol., 1907, **4**, 804.
[32] Bull. méd., 1907, **21**, 952.
[33] Gaz. hebd. d. sci. méd. de Bordeaux, 1907, **28**, 603.
[34] J. de physiol. et path. gén., 1910, **12**, 64.

G. SPECIFICITY OF LOCAL TUBERCULIN REACTIONS

If an individual responds negatively to the foregoing tuberculin tests, and if the case is not one of febrile phthisis which has exhausted all its resources of bodily defence, one may assert that a tuberculous infection exists, progressive or latent, localized or disseminated. It is impossible to draw any further definite indications from the fact that one or more of the tests react positively. The reaction does not inform us as to the organs infected, the extent, or severity of the illness. At most, we know in a general way,—although there are many exceptions,—that the reactions occur earlier and more intensely in subjects who are more recently and less seriously infected and have better resistance. In the really ill the reactions ordinarily manifest themselves later and somewhat violently.

Any further information should not be demanded of local tuberculin reactions. What they do give us is already extremely valuable. In the presence of a suspected lesion they enable us to eliminate definitely its tuberculous nature, although a positive response in no wise authorizes us to assert that the tubercle bacillus is the only or even the principal etiological factor. All that we can say is that the individual who presents it is surely the bearer of a tuberculous focus.

Some observers have questioned the absolute specificity of local tuberculin reactions. Thus Fernand Arloing[35] by instilling tuberculin upon the ocular conjunctiva of animals actively immunized against various bacterial toxins, those of diphtheria bacilli, tetanus or typhoid bacilli for example, states that he has seen a somewhat intensive positive ophthalmic reaction produced.

Now, in collaboration of C. Guérin,[36] I vaccinated a series of animals against the most varied toxins and pathogenic bacteria, without ever being able to arouse in them a special sensitiveness to tuberculin, except in the case of rabbits recently injected intravenously with the typhoid bacillus. Even in these cases the conjunctival reddening appeared only inconstantly and did not have that very peculiar deep red color of the caruncle which characterizes the tuberculin reaction.

The specific sensitiveness of animals to local tuberculin reactions

[35] Compt. rend. Soc. de biol., 1908, **64**, 722.
[36] Ibid., 1908, **64**, 889.

is easily demonstrated by experiments here given (Calmette, M. Breton and L. Petit[37]):

1. Rabbits entirely free from tuberculosis (proved later by autopsy) receive into the marginal ear vein a variable dose of tuberculin; 2 mgms., 5 mgms., and 1 centigram (dry purified tuberculin dissolved in physiological salt solution). Sixteen hours later a drop of a 1 per cent solution of tuberculin is instilled into one eye. By the end of three hours injection of the blood vessels of the conjunctiva is present, localized particularly at the inner canthus of the eye and in the nictitating membrane. This reaction, which is very obvious when the tested eye is compared with the noninstilled, remains for only two or three hours and then disappears. Forty-eight hours later, when the same rabbits are instilled again in the other eye, some react feebly and tardily (after 6 or 12 hours), others not at all. On the third day not one shows any reaction. In each instance, the controls which did not receive tuberculin intravenously, are tested and exhibit no conjunctival reddening.

2. Other rabbits receive intravenously one centigram of bovine bacilli. They are all afterward tested successively every 24 hours by instilling tuberculin into one eye. After the third day a mild positive reaction appears. In the following days it becomes more distinct. It ceases to manifest itself after 16 to 18 days, at the period when the loss of weight indicates that the lesions are already very extensive. The same phenomenon is produced in tuberculous man.

P. Nobécourt and Ch. Mantoux[38] made a comparative study of the ophthalmic and cuti-reactions, after varying periods, in rabbits inoculated subcutaneously, intraperitoneally and intravenously. In their experience, when the cuti-reaction showed itself constantly negative the ophthalmic reaction was inconstant. At times the cuti-reaction was positive, then disappeared to reappear again a little later in the same animal. It was more regular in animals with less extensive lesions than in those more gravely infected, and in no case did it reveal itself before the nineteenth day.

H. Wildholz,[39] after having proved the absence of any reaction in 20 normal rabbits, infected them by the bladder route with bacilli

[37] Compt. rend. Soc. de biol., 1907, **63**, 296.
[38] Ibid., 1907, **63**, 382.
[39] Berl. klin. Wchnschr., 1908, **45**, 545.

of human and bovine origin. Eight weeks afterward, 19 reacted positively to the cuti- and ophthalmo-reaction.

G. Moussu and Ch. Mantoux likewise obtained positive intradermic reactions in all the cattle, swine and goats which they had artificially tuberculized.

In so far as naturally infected animals are concerned, no one longer denies the specificity of local tuberculin reactions. Sometimes, and we have already had occasion to discuss this question in studying bovine tuberculosis (*Chapter XXIV*), one of the tests may be positive and the other negative when made simultaneously in the same subject.

A positive intradermic or ophthalmic reaction has been seen, for example, in cattle which reacted negatively to the cutaneous test. This does not prove that the cuti-reaction is less delicate than the ophthalmic or the intradermic, since outside factors (fault of operative technique, rubbing, etc.) may have intervened to prevent absorption of the tuberculin. But if one of the tests gives a distinctly positive result, one has enough evidence to make a definitely positive diagnosis.

Vallée, Lignières, Klimmer and Kiessig, Voltz,[40] Trotter, and others, in testing herds of cattle with the ophthalmo-reaction, found it to agree with the autopsy findings in every case, with a few very rare exceptions.

In the tuberculous guinea pig, Römer, R. Kraus and R. Volk[41] have observed that the intradermal injection of 0.02 gm. of raw tuberculin gives rise always to a red papule which is distinct after 24 to 48 hours and persists generally for 4 or 5 days, whereas normal guinea pigs give no reaction.

The lower monkeys (*Macacus cynomolgus, sinicus, rhesus*) are, according to Et. Burnet,[42] quite insusceptible to superficial inoculations or to instillations of tuberculin (cuti, intradermic and ophthalmic). On the contrary the reactions are very marked in *chimpanzees*.

In human and veterinary pathology the specificity of local tuberculin reactions is certainly not open to question. They have made it possible to institute a sort of inventory of the distribution of tuberculous infection throughout the world (*see Chapter XL*) and to

[40] München. tierarztl. Wchnschr., 1909, **53**, 153.

[41] Ztschr. f. Immunitätsforsch., 1910, **6**, 683.

[42] Compt. rend. Soc. de biol., 1912, **73**, 248.

determine the ratio of infected to healthy individuals in each large population, in each social grouping, and in each family. Even in this respect alone they have already rendered the greatest service and they will continue to do so in the future when we shall have learned how to use them better.

Thanks to them, we can fix the period in the life of each individual when tuberculous infection first establishes itself in the body and we shall consider somewhat further on the extremely important consequences, from the point of view of individual and public prophylaxis, arising from this determination which in the future is possible, easy and accurate. From the standpoint of the safeguarding of children we shall benefit from such findings in particularly large measure.

H. SIMULTANEOUS OR SUCCESSIVE APPLICATION OF THE VARIOUS TUBERCULIN REACTIONS.—LOCAL SENSITIZATION TO TUBERCULIN

Under certain circumstances where a first tuberculin test has been doubtful, the clinician may be interested to repeat the reaction in the same manner or in a different form, or by applying several methods simultaneously.

It is then important to realize that the subcutaneous injection carried out *at the same time* as the local reactions, prevents the development of the intradermic reaction, that it prevents, diminishes or delays the cuti-reaction, but that it does not interfere with the ophthalmic reaction.

It should be known too that a subcutaneous injection performed before the local reactions, inhibits the intradermic reaction and the cuti-reaction for 2 to 3 days, but that it neither impedes nor retards the ophthalmic reaction.

The local reactions, on the contrary, have no influence upon the general thermic reaction induced by subcutaneous injection. Yet cases have been cited, in cattle, where the intradermic reaction is said to have prevented a later general reaction (Lignières[43]).

The inhibitory effect of the general reaction has been explained by experiments which I performed with M. Breton and G. Petit,[44]

[43] Bull. Soc. centr. de méd. vétér., 1909, **63**, 91.
[44] Compt. rend. Soc. de biol., 1907, **63**, 296.

according to which, when the body is sensitized with a weak dose of tuberculin, the tuberculin reactions are positive, while they are no longer so when the organism is saturated with tuberculin. This last phenomenon is met with in the subcutaneous injection which causes a relatively considerable amount of tuberculin to enter into the body fluids. It will be understood that this dose, which is massive as compared with those used for local reactions, prevents more or less the manifestation of the latter when they are tried simultaneously with, or after, the subcutaneous test. The ophthalmic reaction alone furnishes an exception to this rule because of the extreme susceptibility of the conjunctival mucous membrane, and this property enables one to settle the doubtful result of an earlier subcutaneous injection.

There is still another fact which the clinician should not fail to appreciate if he desires to produce a general reaction or to administer tuberculin subcutaneously for therapeutic purposes in subjects who have been previously subjected to local reactions; namely, that these local reactions may reappear sometimes several weeks after the primary local reaction has been obtained.

This phenomenon of the recurrent reaction (*reviviscence*) was first noted by Slatineanu[45] in man, and by my collaborator C. Guérin[46] in cattle. It has been observed since then by a number of authors (Lenhartz, Moro, Bandler and Kreibich, Fritz Lévy, Danielopolu, J. Lemaire[47] and others). It is almost constant for cuti-reactions performed about one week before, and it often happens that the cutaneous response induced by a general reaction, is more intense than the original cuti-reaction.

With M. Breton and G. Petit,[48] I drew attention to what we termed the "ophthalmo-reaction seconde" which is observed in certain patients ill with non-tuberculous infections. After having failed to react to an intraconjunctival instillation of tuberculin, they suddenly develop a redness of the conjunctiva and caruncle some hours after a subcutaneous injection of 2 mgms. of tuberculin one week later. At the end of 24 hours the redness has disappeared. We have here to do with a phenomenon of local sensitization which

[45] Revista med. Bucarest., 1907, June.
[46] Rec. de méd. vétér. d'Alfort, 1907, July 30.
[47] Thèse, Paris, 1909.
[48] Compt. rend. Soc. de biol., 1907, **63**, 296.

must not be mistaken for a positive reaction. It is moreover only exceptionally that it is reproduced a second time following a further injection some days later.

An experiment performed by L. Massol[49] shows how very susceptible to tuberculin are organs which have been at all infected by the bacillus. He prepares a certain number of animals which receive into the right eye for example, 1 mgm. of bacilli suspended in 1 drop of physiological salt solution.

The following week the animals are injected with 0.01 cc. of tuberculin subcutaneously. Three hours later all are weeping from the right eye, while the left eye preserves its normal appearance. This experimental recurrent reaction "reviviscence," which is evidence of the seat of primary infection, is extremely clean cut.

In the opinion of Wolff-Eisner, the revivifying of local reactions in the course of tuberculin therapy indicates that one has injected too large a dose of tuberculin.

Von Pirquet[50] had likewise noted that repeated cuti-reactions in the same individual increased at times in intensity and even became positive when the first result had been negative. The impregnation of the tissues with tuberculin may therefore induce a peculiar sensitiveness to this substance, and this phenomenon may, in the interpretation of local reactions, lead to errors against which one must be on guard. It seems however, that, in persons as in animals *absolutely free* from any tuberculous infection, the repetition of superficial inoculations or instillations of tuberculin, even though concentrated, *never* gives a reaction. Such at least is the inference from a large number of observations made upon nursing infants and adults who at no time gave a positive ophthalmo-reaction. Roepke[51] never succeeded in sensitizing normal subjects with 4 per cent Koch's tuberculin and by very frequently repeating the instillation into the same eye.

The conclusion from what precedes is that the repetition of local reactions renders them more sensitive and more precise, but that they should not be repeated except in cases where it is desired to eliminate every suspicion of tuberculous infection.

[49] Compt. rend. Soc. de biol., 1913, **74**, 1260.
[50] Wien. klin. Wchnschr., 1907, **20**, 1123.
[51] Beitr. z. klin. d. Tuberk., 1908, **9**, 353; **11**, 245.

I. COMPARISON OF CLINICAL RESULTS OBTAINED WITH THE DIFFERENT LOCAL TUBERCULIN REACTIONS.—PROPORTION OF POSITIVE REACTIONS IN APPARENTLY NORMAL SUBJECTS

Wolff-Eisner[52] studied the cuti- and the ophthalmo-reaction side by side. In apparently healthy subjects the cuti-reaction gave him 50 per cent of positive results; the ophthalmo-reaction but 18 per cent.

In 7 cases where both tests had been negative, there was no trace of tuberculosis at autopsy. In 7 others who had given a positive cuti-reaction and a negative ophthalmo-reaction, autopsy showed complete absence of lesions in one instance; old foci encapsulated or apparently healed in 5 cases; and recent lesions in a cachetic patient in one instance.

Hammerschmidt[53] tried the two methods simultaneously in 500 soldiers in hospital, among whom were some tuberculous cases, both definitely proved and suspected. The ophthalmo-reaction was never positive without the cuti-reaction being so at the same time; but the latter was often positive when there was no conjunctival reaction. He obtained 140 cuti-reactions as compared to 97 positive ophthalmic tests.

Bing,[54] trying the two reactions on non-tuberculous children, found 18.2 per cent of cuti- and 1.2 per cent of positive ophthalmo-reactions.

Of 192 non-tuberculous persons tested by Stadelmann,[55] there were 50 per cent which reacted to the cuti- and 18 per cent to the ophthalmo-reaction.

According to Wolff,[56] the proportion of positive results in suspected individuals, as regards the 3 tests, cuti, ophthalmo and subcutaneous, is the following:

	per cent
Cuti	55.5
Ophthalmo	30.0
Subcutaneous	86.0

Testing several hundred insane adults, Raviart[57] finds 43 per cent

[52] *Die Ophthalmo- und Kutan Diagnose der Tuberkulose.* Wurzburg, 1908.
[53] Med. klin., 1908, **4**, 869.
[54] Berl. klin. Wchnschr., 1908, **45**, 546.
[55] Deutsch. med. Wchnschr., 1908, **34**, 227; 271.
[56] Berl. klin. Wchnschr., 1908, **45**, 295.
[57] Thèse, Paris, 1907.

who give a positive ophthalmo, while Mézie, in another asylum, obtains 87.7 per cent of positive cuti-reactions.

Ch. Mantoux[58] performed the ophthalmo-reaction upon 200 healthy children under public charge, aged from 2 to 16 years, and the intradermic reaction in 300 others. In a similar class group at Vienna, von Pirquet performed 693 cuti-reactions. The following are the percentages of positive reactions obtained by the two observers:

OPHTHALMIC IN 200 HEALTHY CHILDREN (MANTOUX)	INTRADERMIC IN 300 HEALTHY CHILDREN (MANTOUX)	CUTI IN 693 HEALTHY CHILDREN (VON PIRQUET)
per cent	*per cent*	*per cent*
From 2 to 5 years.. 4	From 2 to 4 years...51	From 2 to 4 years...13
From 6 to 10 years.. 9	From 5 to 7 years...66	From 5 to 6 years...17
From 11 to 16 years..10	From 8 to 15 years...84	From 7 to 10 years...35
		From 11 to 14 years...55

Raymond Letulle[59] collected in his thesis the statistical results published by 67 authors and bearing upon 10,485 observations.

They apply to the ophthalmic reaction only. The summary is as follows:

Percentage of positive ophthalmic tests in persons:

Proved definitely tuberculous	88.2
Suspected	56.7
Clinically healthy	15.8

On the other hand, in collaboration with V. Grysez at the Pasteur Institute at Lille, he tested 2108 subjects of all ages with the cuti-reaction. The individuals were taken at random from all classes of the city, among those not in the habit of frequenting either hospitals or dispensaries. The investigation was continued over three consecutive years. The results were the following:

AGE	NUMBER OF SUBJECTS TESTED	PERCENTAGE OF POSITIVE REACTIONS
		per cent
Up to 1 year	405	5.6
From 1 to 2 years	280	20.0
From 2 to 5 years	237	55.0
From 5 to 15 years	446	77.0
Older than 15 years	740	89.0

[58] Congr. on Med., Paris, 1907.

[59] Thèse, Paris, 1912.

Among the children under 1 year who gave a positive reaction, there was one of 2 months and its mother was phthisical. All the others were from 6 to 12 months old.

It is seen therefore that in the city of Lille, 20 per cent of the children are already infected at the age of 2 years; that at 5 years the proportion amounts to 55 per cent; from 5 to 15 years to 77 per cent; beyond 15 years to 89 per cent.

It is evident that, in this immense number of individuals who are detected as bearers of tubercle bacilli by general or local tuberculin reactions there are relatively very few who become phthisical or who succumb to other grave forms of tubercle bacillus infection. If we are to generalize for the whole city of Lille on the strength of the above collected figures, we may grant that 60 per cent of the population of 220,000 inhabitants, in other words 132,000 persons of all ages, harbor tuberculous lesions which are benign or serious, latent or in process of evolution. Now, of the 132,000 persons who are infected with the bacillus, there die each year an average of only 18 per cent from pulmonary tuberculosis, meningitis or other forms of tuberculosis classified in the statistics of the Bureau of Hygiene (736 deaths from all forms of tuberculosis of a total of 4083 deaths in 1912). The immense majority of them therefore tolerate their bacilli and, in spite of the presence of the latter in their bodies, preserve the appearance of more or less robust health, or else die of a great variety of diseases, the latter however being often aggravated by the preëxisting tuberculous infection.

K. Franz,[60] an Austrian army physician. tested 400 young soldiers of a Bosnian regiment by the subcutaneous method. Of this number, 245, or 61 per cent, reacted to doses below 5 milligrams; 10, or 2.5 per cent, gave a doubtful reaction, and 145 or 36.5 per cent gave no reaction.

In a second series of Bosnian recruits made up of 323 individuals 222, or 68.8 per cent, reacted. In comparison, 279 recruits of another regiment composed chiefly of Hungarians were submitted to the same test; 108 gave a positive reaction, or only 38.7 per cent.

During their 3 years of active service and their 3 or 4 years of service in the reserve, about 7 years altogether, all of these men could be kept under more or less close observation. From the first

[60] Wien. med. Wchnschr., 1902, **52**, 1689;—Wien. klin. Wchnschr., 1909, **22**, 991.

series of 400 Bosnians, 32 (8 per cent) became clinically tuberculous: of the second series of 323 Bosnians, there were 23 (7.12 per cent), and of the series of 279 Hungarians there were only 9 (3.22 per cent) who developed clinical disease.

Of the total of 1002 recruits examined by Franz there were 575, or 57.38 per cent, who had reacted to tuberculin. Of these 575 "carriers of bacilli" or bearers of latent infection, 64, or 6.38 per cent became clinically tuberculous during the course of their 7 years of military service.

The rural population, in spite of what one might think, is not spared as regards its children, even in the parts of Europe where tuberculosis is least frequent. Hillenberg[61] tested 810 healthy children in a locality in Germany where the disease is very rare. He found the following percentages of positive reactions:

From 6 to 10 years	19.8
From 11 to 15 years	31.5

In France, Et. Burnet[62] began an investigation in a rural community near the sea and where tuberculosis is uncommon, with a view to determining the moment at which infection appears in children. The study bears upon 77 subjects, 62 of whom he was again able to see after two and one-half years. His summary gives the following results:

	NUMBER OF CHILDREN	POSITIVE REACTION	PER CENT
1st year	7	0	
2nd year	5	0	
3rd year	5	0	
4th year	8	0	
5th year	12	1	8.3
6th year	12	2	16.6
7th year	10	4	40.0
8th year	2	1	
10th year	1	1	
	62	9	14.3

[61] Ztschr. f. Hyg., 1909, **64**, 305;—Tuberculosis, 1911, **10**, 254.
[62] Ann. de l'Inst. Pasteur, 1915, **29**, 274.

In a nursery at Hamburg, receiving chiefly illegitimate children with their mothers, Moltrecht[63] found among 26 nurslings in the first year 11 positive reactions (of whom were 9 from 1 to 7 months old), and among 17 in their second year, 8 positive reactions. Here the proportion is enormous.

Obviously it is in tuberculous families that one finds the largest number of positive tuberculin reactions among children still healthy in appearance. The infection exists moreover in its very early stage and the cuti-reaction, which can be performed so easily, permits its detection. In this wise Pollak at Vienna examined the children of 200 families in which one or more members were affected with tuberculosis. He found the reaction negative in only 9 instances. Children born after the disease had appeared in the parents were all infected.

Further statistical evidence would seem unnecessary. In the foregoing statements, the information to be gleaned from local tuberculin reactions has been made sufficiently clear. Their technique is very simple. That of the cuti-reaction particularly is without pain, is devoid of danger and can be employed at all ages. With adolescents and adults these reactions are of but little interest because of the extreme diffusion of the tuberculous infection. Too frequently they are positive and their capacity for revealing apparently healed tuberculoses, as well as forms which are latent or slowly progressive, makes them of use only when it is necessary to eliminate tubercle bacillus infection from the diagnosis. On the other hand, *in young children*, and above all *in nursing infants*, they are tremendously valuable as *evidence of a recently acquired infection.*

[63] Beitr. z. klin. d. Tuberk., 1914, **31**, 275.

CHAPTER XXXVII

TUBERCULOUS "ANTIBODIES" AND THEIR RÔLE IN THE DEFENSE OF THE BODY AGAINST INFECTION

A. ANTIGENIC FUNCTIONS OF TUBERCLE BACILLI AND OF THEIR SECRETORY PRODUCTS.—TUBERCULOUS ANTIBODIES.—THEIR DEMONSTRATION BY THE FIXATION REACTION OF BORDET-GENGOU

When introduced into the body of an animal either susceptible or naturally refractory to tuberculous infection, tubercle bacilli,—like the majority of bacteria which do not cause a rapidly fatal infection or intoxication,—provoke the formation of substances generally antagonistic and today called *sensibilisatrices*, or more commonly *antibodies*. These substances are produced by the leucocytes of the animal and are set free in its body fluids.

The fundamental discovery of antitoxins by von Behring and the still more fruitful discovery, due to Metchnikoff and his pupils, principally to Jules Bordet, of the mechanism of resorption of cellular elements and of albuminoid substances, have shown that therein lies a general phenomenon from which spring the laws of natural or acquired immunity.

The bacteria, the cellular bodies and the albuminoid substances which give rise to the formation of *antibodies* (*sensibilisatrices* of J. Bordet, *amboceptors* of Ehrlich) are called *antigens*.

All the antigens do not possess the same capacity for bringing about the formation of antibodies and each animal organism reacts in its own peculiar manner to such an antigen, so that the production of antibodies is a matter of endless variety.

The function of these antibodies is at times of a defensive nature; such is the case with the *antitoxins* which are the *antibodies of the bacterial poisons; of vegetable toxalbumins* (*ricin*, *abrin*, etc.) *or of the venoms*. But, very often, they exert no protective action. They appear only as evidence of certain processes of cellular digestion, and it seems indeed that such is the case in tuberculous infection.

The interest in the search for them and in their study is nevertheless considerable, since they give information which is very useful in the diagnosis and prognosis of this infection.

The principle of the fixation reaction of Bordet-Gengou

The demonstration of antibodies in the serum or in any other body fluid can be accomplished by the so-called reaction of fixation of Bordet-Gengou.

The underlying principle of the reaction is the following:

When an antigen and the corresponding antibody are brought together, the alexin (or complement) of a fresh serum fixes itself to the antigen. The proof of this phenomenon is furnished by the fact, that, *if goat red cells*, for example (previously rid of every trace of serum by several successive washings and centrifugations), are added to the mixture together with a suitable quantity of *serum hemolytic for the goat red cells* and *inactivated by heating at* 58°C., the complement being no longer free to *activate* this hemolytic serum, is incapable of *fixing itself to the red cells* and of producing hemolysis.

The absence of hemolysis serves as evidence of the *deviation* or, in other words, of the *fixation of complement* to the complex *antigen + tuberculous antibody.*

If the same test is carried out by combining antigen and complement alone or antibody and complement alone, fixation is not possible, and the complement remains *free.* It can then in the presence of goat erythrocytes and of inactivated hemolytic antigoat serum activate the latter and hemolysis takes place.

This reaction of Bordet-Gengou is employed in the search for antibodies in a large number of infectious diseases. The so-called Wassermann reaction is a successful application to the diagnosis of syphilis. F. Widal and Le Sourd, Camus and Pagniez[1] were the first to attempt to take advantage of it in tuberculous infection in man, and Bordet, in collaboration with Gengou,[2] in experimental infection. Since then a great number of laboratory workers and clinicians have used it with often inaccurate or discordant results, and we know today that this lack of agreement is due to the fact that the technique, which is a delicate one, was poorly defined.

[1] Compt. rend. Soc. de biol., 1901, **53**, 734.
[2] Compt. rend. Acad. des sci., 1903, **137**, 351.

B. THE PREPARATION OF WASHED RED CELLS.—PREPARATION AND TITRATION OF HEMOLYTIC SERA.—CHOICE, TITRATION AND PRESERVATION OF COMPLEMENT

For carrying out the fixation reaction there must be a preliminary preparation and titration of the various elements which enter in.

These elements are, in addition to a good *antigen* and the *serum to be studied,* in which the presence of antibodies is sought:

1. A *suspension of washed red blood cells;*
2. An *inactivated hemolytic serum,* whose hemolyzing power is known;
3. A *titrated complement.*

1. Washed red cells

Usually one employs the blood of a sheep or goat collected in a sterile flask about one-eighth filled with glass beads. The flask is shaken until the fibrin is separated and the supernatant blood remains fluid. The defibrinated blood is poured into centrifuge tubes in order to wash the red cells as completely free from serum as possible. Each tube is filled about one-quarter full with blood, marking the level with a pencil, and then three-quarters with sterile physiological salt solution (0.85 per cent NaCl). The red cells are thrown down by centrifugation. The supernatant fluid is poured off and replaced by physiological salt solution. The red cells are again taken up in suspension and centrifugated. The operation is repeated 3 times. After the third washing a quantity of physiological salt solution is added to the tube in an amount equal to the original volume of whole defibrinated blood. The suspension of erythrocytes thus obtained is finally diluted half and half with physiological salt solution and is ready for use.

The suspension can be preserved as it is for 2 or 3 weeks in an ice box at a temperature of + 2° to + 5°, in sterile tubes plugged with cotton, or else, as Armand-Delille has suggested, by immediately adding a solution of formalin (40 per cent formaldehyde) in a proportion of 1 to 500.

2. Hemolytic serum

In order to obtain a good *hemolytic serum specific* for red cells (of sheep or goat) to serve for the test, 1 cc. of these red cells, washed

as described above, is inoculated aseptically under the skin of a rabbit. The injection is repeated with 2 cc. after five days and a third time with 2 cc. after still another five days.

Five days after the final injection, the animal is bled from the carotid or femoral artery; the blood is allowed to coagulate aseptically and the serum collected. The latter contains both *anti-sheep* and *anti-goat* hemolysins (so that the red cells of sheep or goat may be employed indifferently). It is portioned out in small tubes which are sealed in the flame and heated a half hour in a water bath at 58°C. in order to effect *inactivation*, that is to say, in order to *destroy the complement* contained therein. One of the tubes is to serve for the titration, which should be carried out in the following manner:

Determination of hemolytic activity. One cubic centimeter of hemolytic serum is diluted with 99 cc. of physiological salt solution and added successively to each of a series of test tubes in amounts of 0.1 cc., 0.2 cc., 0.3 cc., etc., up to 1 cc. or more if necessary. All tubes are made up to the same volume (2 cc. for example) with physiological salt solution. To each of them is added 0.1 cc. of fresh guinea pig serum (complement), then one drop of a suspension of washed sheep or goat red cells prepared as already described.

All the tubes are made up to 3 cc. with physiological salt solution, with care to rotate them somewhat between the fingers in order to wash down the sides. Finally they are left in the incubator at 37°C. for one hour.

If hemolysis begins to take place in the tube containing 0.5 cc. of the dilution of hemolytic serum and is complete in all tubes where the serum is present in larger amount, it is said that this serum hemolyzes in a dose of 0.005 cc. in the presence of 0.1 cc. of fresh guinea pig complement.

In every fixation reaction it is necessary to use an amount of hemolytic serum equal to at least 10 or even 20 times the minimal hemolyzing quantity. The least trace of complement remaining free will then manifest its presence by dissolving the red cells, which would have been left intact with a smaller dose of hemolytic serum. A hemolytic serum of which the minimal hemolytic amount is found to be 0.005 cc. will therefore be employed constantly for each tube in a quantity of 0.1 cc. (undiluted).

Stocks of such hemolytic serum may be preserved for a long time (several months) in an ice-box in sealed tubes or in well corked flasks.

The strength falls off only slowly. It will however be well to titrate anew from time to time.

3. *Complement*

The best complement is that furnished by fresh guinea pig serum. The serum can be obtained by centrifugating defibrinated blood collected aseptically by puncturing the heart or by bleeding from the carotid.

In all cases complement must be *titrated* before use in the tests, inasmuch as its strength varies with different guinea pigs.

It must be realized moreover that it loses one half of its original titer if allowed to remain for one hour in a dilution of 1 in 20 (the time required for the fixation reaction), as L. Massol and V. Grysez[3] have shown.

The same authors carefully studied the effect of age upon the strength of complement. By preserving 10 guinea pig sera in an ice-box (at 4° to 6°), they found that the complement strength falls rapidly after the second day. By the ninth day only one had retained 50 per cent of its original strength; the others only 12 per cent to 37 per cent. After 16 days they had lost 85 to 90 per cent of their strength and were therefore practically useless.

On the other hand, certain unpublished experiments of L. Massol which were made in my laboratory and which I was able to follow, show that it is easy to preserve complement for a long time in a refrigerating apparatus at a temperature of −10° to −15° (for example the Audiffren apparatus, of Singrün, which is easily employed).

During the first month the loss of complementing strength of fresh guinea pig sera preserved under these conditions, varied from 30 to 50 per cent. But after 9 months the same sera were found to have retained 20 per cent to 33 per cent of their original titer.

L. Massol and Nowaczinski called attention to still another procedure which appreciably retards the loss of complementing activity. It consists in adding to fresh serum one-tenth of its volume of a saturated aqueous solution of NaCl (36 gms. per liter). This amount of salt offers no inconvenience since in carrying out fixation reactions the serum is diluted with physiological salt solution. Dilution need only be made, at the moment of use, with *distilled* water, in order to restore normal tonicity to the serum. By this method the titer of

[3] Compt. rend. Soc. de biol., 1910, **68**, 588.

complement remains unmodified for about 10 days. After 18 days it still retains 75 per cent of its strength and after 25 days, 25 per cent of its strength.

The foregoing statements indicate how *very important are preliminary titrations of complement.* Many workers neglect this and it follows that their results are often erroneous.

It is therefore necessary to determine with the greatest care the *minimum active dose,* variable with each complement, which will permit the detection of the smallest traces of antibody or hemolysin. It should also be recalled that during a period of one hour when diluted to a volume of 2 cc.,—that it to say during the time necessary for fixation,—this minimum dose is found to have about doubled (Calmette, L. Massol and V. Grysez).

Titration of complement. A 1 in 100 dilution of guinea pig serum is made up with physiological salt solution with 8.5 gms. of NaCl per liter. Into a series of small test tubes are introduced 0.1 cc., 0.2 cc., 0.3 cc., etc., up to 1 cc. of the diluted complement (or of larger doses if one is using old complement). Physiological salt solution is added to all tubes up to 2 cc. To each tube are added 20 minimal hemolytic units of inactivated hemolyzing serum, then one drop of a 5 per cent suspension of washed red cells, and the volume is made up to 3 cc., always with physiological salt solution. The tubes are left in the incubator for one hour at 37°C.

The amount of complement remaining free (not fixed) is indicated by the first tube of the series where hemolysis is found. *Twice this amount will be taken as the minimal quantity in the fixation tests.*

To avoid using complement in too great quantity, a dilution should be made so that 0.1 cc. shall contain the minimal dose. With fresh guinea pig complement the dilution to be made varies in general from 1 in 6 to 1 in 10.

C. PREPARATION OF SERA TO BE TESTED AND TO BE TITRATED FOR ANTIBODIES

If the blood of a patient or of a subject suspected of tuberculosis is to be tested for antibodies it should be taken either by venous puncture with a sterile syringe or by scarifying and cupping.[4] In all

[4] Mézie has had a special form of cup made for this purpose, provided with a reservoir to collect the blood and with an outlet through which a vacuum is created with an air pump or Potain aspirator. (Compt. rend. Soc. de biol., 1911, **70**, 30.)

cases, 15 to 20 cc. of blood are necessary to obtain 5 to 8 cc. of serum. The latter should be prepared by defibrinating immediately and centrifugating, or by allowing the clot to form and pouring off the serum after 18 to 24 hours. It is collected in sterile pipettes which are sealed in a flame and inactivated for 30 minutes in a water bath at 56°C. to destroy the complement.

Serum thus prepared can be preserved for several days and even for weeks in an ice-box without its antibody content being modified.

The sera of tuberculous or suspected animals should be collected under the same conditions and in like manner freed of complement by preliminary heating at 56 or 58°C. for a half hour.

However, if one is trying to determine the amount of antibody in sera which can contain but very small quantities of it, for example in that of patients who have not received injections of tuberculin or in that of small laboratory animals into which weak doses of antigen have been injected, it is necessary to dispense with the preliminary heating at 58°C. which always destroys an appreciable proportion of amboceptor. It is therefore recommended to preserve the samples of serum to be studied for 10 days at laboratory temperature, provided they remain absolutely sterile. Under these conditions the normal complement disappears almost entirely and what remains may be disregarded, if the dilutions for the fixation reactions are made sufficiently high.

D. CHOICE AND PREPARATION OF ANTIGENS.—MEASURE OF THEIR VALUE.—THEIR RELATIVE STABILITY AND SPECIFICITY

Either tubercle bacilli or various substances derived from them may be used as antigens, for example, tuberculin or the products extractable from the bacterial bodies; but the tissues, even though containing many miliary tubercles, and also the waxes or fats derived from the bacilli and separated from the bacterial bodies, are not suitable (E. Grancher, H. Salin and G. Bricout,[5] K. Bierbaum and G. Berdel,[6] M. Burger and B. Mollers,[7] although Hammers[8] claims to have used them advantageously in the form of alcoholic or acetone extracts).

[5] Compt. rend. Soc. de biol., 1912, **73**, 439.

[6] Ztschr. f. Immunitätsforsch., 1914, **21**, 249.

[7] Deutsch. med. Wchnschr., 1916, **42**, 1573.

[8] München. med. Wchnschr., 1912, **59**, 1750:—Deutsch. tierarztl. Wchnschr., 1912, **20**, 593.

The qualities of an antigen depend:

1. On its ability to bring about the formation of the greatest possible quantity of antibody in the living organism;

2. Upon its affinity for tuberculous antibodies when combined *in vitro* with the latter and a proper quantity of complement.

No antigen seems to possess these two qualities to the highest degree at one and the same time.

K. Momose[9] used as antigen a special tuberculin which he calls T.A.C., obtained by treating with sodium hydroxide bacilli which have been freed of fat by extraction with chloroform. He prepares it in the following manner:

Bacilli from broth cultures 6 to 8 weeks old are washed with physiological salt solution, then with distilled water on a filter, then pressed between two double layers of blotting paper and weighed. They are carefully ground in an agate mortar, a 10 per cent solution of sodium hydroxide being added drop by drop meanwhile until there are 10 cc. of solution to 1 gm. of bacilli. The suspension is then poured into an Erlenmeyer flask which is shaken continuously for 48 hours. After this treatment it is centrifugated. The saponifiable fats pass into the supernatant fluid which is poured off. The sediment is taken up with a small quantity of physiological salt solution and supplemented with a considerable excess of chloroform after which it is again shaken continuously for at least two hours. Then after centrifugating anew, the chloroform layer with its chloroform-soluble lipoids is withdrawn with a pipette. The supernatant fluid contains the substances which will make up the antigen.

The chloroform extraction with shaking and centrifugation is repeated and, after a final decantation, a current of filtered air is passed into the fluid in order to remove the traces of chloroform.

The volume of fluid is finally measured and made up with physiological salt solution so that 10 cc. of emulsion corresponds to 1 gm. of the originally employed bacilli (weighed moist and therefore containing about 75 to 80 per cent of water).

The emulsion is cloudy, grayish in color, and has the odor peculiar to tubercle bacilli. It can be dried in a vacuum to facilitate preservation. It contains débris of bacilli which have almost entirely lost their acid-fastness.

[9] Veröffentl. d. R. Koch Stift. z. Bekämpf. d. Tuberk., 1913, H. 8/9, 42.

This mode of treatment eliminates an amount of lipoids varying from 9.78 to 13.04 per cent of the dry weight of the human bacilli employed. The bovine bacilli furnish perceptibly more lipoids than the human.

With this antigen the formation of antibodies can be brought about in all small laboratory animals, particularly in the rabbit. On subcutaneous injection it is well absorbed and does not produce abscesses. For the rabbit the initial dose of dry product is 10 mgms. It is raised successively about every 5 days to 20, 30, 50, and 70 mgms.

Good results are obtained also by intravenous injections of 5, 10, 15, 30 and 50 mgms.

The highest antibody content of the serum is observed during the second or third week after the last injection. The *unheated* serum in which the antibodies are to be titrated should, according to the technique adopted by Momose, be diluted with physiological salt solution in such a way that each fixation test is upon a series of tubes of which every one shall contain a diminishing quantity of serum, successively 0.1 cc., 0.05 cc., 0.025 cc., 0.0125 cc., 0.00625 cc., 0.003125 cc., 0.0015625 cc., etc.

The T.A.C. antigen should be diluted to 1 in 10,000 or even 1 in 20,000 on a basis of dry weight, that is 1 centigram in 100 or 200 cc. of physiological salt solution. For each tube 0.5 cc. should be employed.

The test is carried out in the following manner:

	TUBES						
	I	II	III	IV	V	VI	VII
Physiological salt solution..........	0.5 cc.	0.5	0.5	0.5	0.5	0.5	0.5
Specific serum (in dilutions)........	0.2 cc.						
T.A.C. antigen (diluted 1 in 10,000).	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Complement (diluted 1 in 10)......	0.5	0.5	0.5	0.5	0.5	0.5	0.5

The tubes are put in the incubator for one hour at 37°C., after which there is added to every tube 1 cc. of *sheep red cells.* These cells should have been sensitized through having been treated for one hour at laboratory temperature with an excess of inactivated *anti-sheep* hemolytic serum, then centrifugated, washed in physiological salt solution, re-centrifuged and suspended in a quantity of physiological saline solution corresponding to the original total blood volume from which they were derived.

Results are read after one hour at laboratory temperature.

When a rabbit, after having undergone a series of injections of antigen, is tested after two to three weeks, 0.003125 cc. (tube VI) of its serum, often only 0.0015625 (tube VII) or even less, suffices to produce the fixation reaction under the above indicated conditions. Later bleedings furnish a serum less and less rich in antibodies; but if the animal is left untouched for 3 to 4 months it can be restimulated by a new series of 4 or 5 injections and its serum becomes once more very active.

Of course, with each test it is necessary to run *controls of the quantity of antigen adopted* with a *serum taken from a normal animal* at the same time that the specific serum is taken from the treated animal.

As a general rule, in my own experience, *antigens prepared from bacilli, whether rid or not of their fatty waxy material but also as little modified as possible by chemical reagents*, are to be preferred to dead or living bacilli for the Bordet-Gengou reaction. The essential is that these antigens shall contain only the smallest possible quantity of free lipoids, since the latter unquestionably impede or prevent the fixation of complement *in vitro*.

On the other hand, for the purpose of obtaining antibodies *in vivo*, dead bacilli *and above all, living bacilli*, constitute by far the best antigens.

The richness of a culture in antigenic substances varies greatly with the age of the culture and the chemical composition of the medium upon which it has been grown. From curves plotted by R. Möllers,[10] the increase on glycerin broth or on an asparagin medium without peptone is progressive up to the fifth or sixth week and falls notably from then on. It is said to be, in a certain measure, independent of the weight of the bodies of the bacilli.

I have found with L. Massol that various sera, principally those from *hyperimmunized* animals, do not reveal their antibodies except when combined with antigens obtained by macerating bacilli in peptonated water, after the technique which I shall explain shortly.

The raw tuberculin of Koch, used by certain workers in amounts of 0.01 cc. to 0.03 cc. for each reaction, gives results which are both inconstant and inaccurate. It is in fact capable of fixing the antibodies contained in certain normal sera, such as that of the horse,

[10] Deutsch. med. Wchnschr., 1913, **39**, 2460.

or pathological sera, for example in typhoid or streptococcus infections, as has been noted by Y. Fukuhara.[11]

If the tuberculin is prepared by precipitating with alcohol or by previously separating the bacilli from the culture media before concentrating the liquid, the product obtained ordinarily shows itself incapable of serving as antigen. It appears therefore that, in the case of raw tuberculin, the substances capable of performing the antigenic function are modified by precipitation with alcohol.

On the other hand, extracts prepared by simple maceration on a water bath (for 48 hours at 65°) of 5 gms. of well washed dried bacilli, and suspended in a liter of distilled water, then filtered and concentrated in vacuo to 100 cc., represent an antigen which we call B^1. It is useful for detecting antibodies in certain sera, while those in other sera remain hidden.

If on the contrary, a similar extract of tubercle bacilli is prepared, not with distilled water, but by the maceration (48 hours on a water bath at 65°C.) of 5 gms. of washed bacilli in 100 cc. of a 10 per cent solution of Witte's peptone in distilled water, followed by filtration, there is obtained an excellent antigen which we call B^2. It reveals the most minute quantities of antibody contained in the various sera of tuberculous human beings or hyperimmune animals.

Yet the peptone is inactive in itself, since if it be added in a proportion of 10 per cent to the simple aqueous extract, after the preparation of the latter, the liquid obtained does not detect antibodies in the sera of hyperimmune animals. The peptone therefore, through prolonged maceration with heat, extracts from the bacilli an antigen which is insoluble in pure water. This particularly active antigen, which we have designated B^2 or *peptonated antigen*, is one which we recommend as preferable to all others, on account of its high fixation capacity, its perfect stability, the simplicity of its preparation and also because it enables one to eliminate the intervention of complex substances which for the most part are inactive or simply troublesome and which are contained in the raw tuberculin of Koch.

A. Besredka[12] has proposed using as antigen for fixation reactions, a 30-day old culture of tubercle bacilli grown upon his egg broth, sterilized at 155° and filtered (*Chapter II, A, C*).

[11] Ztschr. f. Immunitätsforsch., 1912, **12**, 183.
[12] Ibid., 1914, **21**, 77.

E. Debains and Jupille,[13] using this egg broth, performed the fixation reaction on the blood of 580 subjects in whom clinical examination was possible.

In cases of pulmonary tuberculosis in the initial stage, presenting only few or doubtful signs, they had 93.4 per cent of positive reactions; in the advanced tuberculous, only 82.3 per cent; in the various tuberculoses without active pulmonary lesions, 96 per cent.

In patients clinically non-tuberculous, the number of positive reactions (among 121 subjects) was 17.3 per cent, and in healthy persons (62) 3.2 per cent.

These authors have noted that syphilis frequently influences the fixation reaction toward the positive, so that when the Wassermann reaction is strongly positive and the clinical examination doubtful, conclusions are to be drawn only with reserve.

They believe that, contrary to the cuti-reaction, the fixation reaction possesses great clinical value and enables one to make a diagnosis of tuberculosis when clinical signs are still concealed or questionable.

From our comparative experiments the qualities of Besredka's antigen are practically identical with those of our peptonated antigen.

The "partial antigens" which Deycke and Much have prepared by treating tubercle bacilli successively with chloride of benzoyl, potassium, and then with 1 per cent lactic acid, do not appear from recent work to be endowed with any particular value either for the diagnosis or treatment of tuberculous infection.

1. Determination of the strength of an antigen

The antigenic strength of a suspension of bacilli, of a tuberculin or of an extract of bacilli, may be determined by means of a type serum containing antibodies, by the following methods (Calmette and Massol):[14]

a. A series of 10 tubes is set up. Each of them receives the same amount of antibody-containing serum, previously inactivated by heating for 30 minutes at 58°C., and varying doses (such as 0.1 cc. 0.2 cc., 0.3 cc., etc. up to 1 cc.) of a dilution of the antigen whose value is to be determined. Each tube next receives a uniform amount

[13] Ann. de l'Inst. Pasteur, 1915, **29**, 182.
[14] Compt. rend. Soc. de biol., 1912, **72**, 15.

of fresh guinea pig serum, for example 0.05 cc. which is 10 minimal doses if 0.005 cc. of this complement represents the minimal dose capable of inducing hemolysis in the presence of a fixed amount of the inactivated hemolytic serum which is to be used. All tubes are made up to 2 cc. with physiological salt solution and placed in the incubator at 37° for two hours (optimum length of time) (L. Massol).[15] At the end of this period, there is added to every tube a uniform quantity of a suspension of washed red cells (of the sheep for example) and 0.1 cc. of an anti-sheep hemolytic serum (of which 0.005 cc. is the minimal hemolytic dose in the presence of an excess of complement). The tubes are again placed in the incubator at 37° for one hour, after which the results are read.

If it is found that there is no hemolysis in the tubes which contain 0.3 cc. and more of the dilution of antigen, while hemolysis is complete in those which contain only 0.1 and 0.2 cc., one concludes that the dose of 0.3 cc. of the dilution of antigen employed fixes 0.05 cc. of complement, or 10 doses of complement of which 0.005 cc. represents the minimal quantity capable of inducing hemolysis in the presence of an excess of inactivated hemolytic serum.

b. The strength of an antigen may also be determined and with more precision by using a single dose of this latter, 0.25 cc. for example, determined by the foregoing experiment, and varying doses of complement (0.01 cc., 0.02 cc., 0.03 cc., 0.06 cc.), allowing all other factors to be constant. The control tubes receive separately antigen alone and amboceptor alone, with the same amounts of complement.

This test gives the number (n) of minimal doses of complement which can be fixed by the volume of antigen employed (v).

To compare one antigen with another, it is enough to establish the ratio $\frac{n}{v}$.

An antigen of which 0.1 cc. deviates 10 minimal doses of complement has for a value $\frac{10}{0.01} \times 1000$. One cubic centimeter of this antigen is capable of deviating 1,000 doses of complement.

Another antigen of which 0.2 cc. fixes 9 minimal units of comple-

[15] Compt. rend. Soc. de biol., 1914, **77**, 140.

ment has a value $\frac{9}{0.02} \times 450$. The latter is therefore 2.22 times weaker than the preceding.

The strength of an antigen, determined in the presence of a known serum and expressed in units of deviated complement, represents a figure which does not vary, provided the hemolytic system (washed red cells and hemolysin) remains constant, a condition easy to obtain.

2. Relative stability and specificity of different antigens

Tuberculous antigens do not have a constant strength; consequently they should be carefully titrated before each series of fixation tests. This is especially true of bacterial suspensions. If the latter are kept at laboratory temperature, their deviating capacity increases for several weeks, particularly during the first days after the suspension has been prepared. If left in the ice-box, they behave contrariwise and rapidly lose a part of their strength.

Extracts of bacilli, especially the peptonated extracts and also the raw tuberculin of Koch, lose their fixative properties but slowly. They can be preserved for months, provided they are kept in sealed ampules and shielded from light.

The specificity of these antigens is not absolute. For example, as J. Bordet and O. Gengou have found, avian tubercle bacilli, the homogeneous bacilli of Arloing, certain paratuberculous acid-fasts like the butter bacillus of Rabinowitsch, the bacilli of Korn, or those of Tobler (I, II and V), may serve as antigens to fix antibodies contained in the sera of animals infected with the human bacillus. Babès and Busila[16] made the same observation with an ethereal extract of the timothy bacillus, while a suspension of these bacilli gives negative results.

L. Massol has demonstrated that diphtheria bacilli, whose chemical constitution somewhat resembles that of tubercle bacilli, bind the antibodies produced by the latter and he has observed that inversely, tubercle bacilli are capable of fixing diphtheria antibodies.

In the opinion of Much and Leschke,[17] the tuberculous antibodies are composed of a mixture of bacillary antialbumins and antifats.

[16] Compt. rend. Soc. de biol., 1910, **68**, 181.
[17] Beitr. z. klin. d. Tuberk., 1911, **20**, 405.

Each of these groups of antibodies is regarded as having a specific action and both of them as being indispensable for the attainment of an antituberculous immunity. This is however an hypothesis which experiments performed with properly purified tuberculous fats or waxes do not justify.

The same applies to the conception of Kurt Meyer,[18] which is contrary to the conclusions reached by Otto Deilmann.[19] Meyer believes that the deviating capacity on the part of tubercle bacilli is due to phosphatids (constituents of these bacteria) which are soluble in benzol, ether and petroleum ether, but insoluble in acetone; whereas the fats and the fatty acids and the waxes are said to play no rôle.

E. ANTIGENIC PROPERTIES OF ORGANS, EXUDATES, PUS AND GLANDULAR EXCRETIONS OF TUBERCULOUS SUBJECTS

S. Bertarelli[20] obtained antibodies in rabbits inoculated with the splenic pulp of tuberculous guinea pigs, but it was essential that this spleen should contain only gray miliary tubercles. With more advanced lesions the result was negative.

It seems certain that the antigenic value (at best very weak and generally nil) of organs and other tuberculous products is in proportion to their content of bacilli. This is proved by fixation experiments *in vitro* performed with extracts of various tissues (lungs, glands, lupus lesions) by Debré and Paraf and by Hammer.

Most frequently these extracts of organs, especially those of glands, pus, sputum and pleuritic or other exudates, contain on the contrary, antibodies, as we shall see later, whereas their antigenic power is nil or very weak.

However, exception must be made for the urine of persons affected with renal tuberculosis. R. Debré and J. Paraf[21] have shown that what they termed the "*reaction de l'antigen*" (which is only the complement fixation test of Bordet-Gengou using a body fluid more or less rich in bacilli as antigen) against a serum of known antibody content is applicable to the diagnosis of certain lesions or certain effusions.

[18] Ztschr. f. Immunitätsforsch., 1912, **14,** 359; **15,** 245.

[19] Ibid., 1911, **10,** 421.

[20] Riv. d'ig. e. san. pubb., 1907, **18,** 97.

[21] Compt. rend. Soc. de biol., 1911, **71,** 65; 169; 359:—Rev. de méd., 1914, **34,** 1; 98.

This reaction can be easily obtained by using 0.4 cc. to 0.6 cc. of urine, which amount in itself is not usually hemolytic. It enables one to state whether an orthostatic albuminuria and certain cases of acute nephritis in children are of tuberculous origin.

R. Debré and J. Paraf consider that various products other than tuberculin and which are said to be capable of passing in the urine may react as an antigen, apart indeed from the presence of bacilli in these urines. They support this hypothesis by a clinical observation published by Alberto Koch and a case reported by Kindberg[22] in which both the reaction of antigen and inoculation of blood into a guinea pig were positive while inoculation of urine remained negative. This interpretation is certainly not correct, since the tubercle bacillus products which are capable of serving as antigen are not dialysable. If, in Kindberg's case, the guinea pig inoculated with urine did not take tuberculosis, it is undoubtedly due to the fact that the amount of urine injected into the animal did not contain bacilli in sufficient number.

F. EXAMINATION FOR ANTIBODIES OR AMBOCEPTORS IN TUBERCULOUS SERA—THEIR TITRATION

When the different elements necessary for the fixation reaction have been prepared and titrated, both the examination for antibodies and their quantitative determination may be patterned after those of the antigens.

For each serum three series of tubes should be set up in the same rack.

The first series, A, comprises 3 tubes, each of which receives a fixed dose of titrated antigen, for example 1 cc. of the peptonated antigen which we call B^2 (Calmette and Massol) diluted 1 in 10.

The second series, B, comprises three tubes, each of which receives 0.5 cc. of the serum to be studied.

The third series, C, is made up of at least 5 tubes. Each receives the quantity of antigen of the first series A and the dose of serum of the series B.

Then, to each tube of all three series, complement is added in increasing amount from tube to tube, beginning with double the amount of the minimal dose producing hemolysis with the combina-

[22] Thèse, Paris, 1913.

tion of red cells + inactivated hemolytic serum; for example 0.01 cc., 0.02 cc., 0.03 cc., and 0.05 cc.

All tubes are made up to 2.5 cc. with 0.85 per cent physiological salt solution, care being taken to revolve the tubes between the fingers in order to wash down any complement which may adhere to the sides.

The rack containing thus the three series of tubes is left in the incubator at 37°C. for one hour; afterward there is added to each tube one drop of a suspension of washed red cells, prepared as previously described, and then an amount of inactivated hemolytic serum equal to 10 or 20 times the minimal hemolytic dose (for example 0.1 cc. of a serum of which 0.005 cc. is the minimal hemolytic dose in the presence of an excess of complement).

All tubes are further made up to 3 cc. and returned to the incubator at 37°C. for one hour. The results are then read.

The complement is deviated in the tubes in which hemolysis is not found. The reaction is *positive,*—therefore indicating the presence of antibodies—if the complement deviated by the mixture antigen + serum to be studied (series C) exceeds the sum of the volumes of complement deviated by the antigen and by the antibody separately (series A and B).

Definition of the unit of antibody. If a volume v of serum to be tested deviates n minimal doses of complement, the proportion $\frac{n}{v}$ represents the number of minimal doses of complement which can be deviated by 1 cc. of serum. It results from this that *the unit of antibody,* like that *of antigen,* may be represented by the quantity of antibody capable of deviating a minimal dose of complement (Calmette and L. Massol).[23]

G. METHOD OF SECURING SERA RICH IN ANTIBODIES

The majority of tuberculous subjects, no matter to what animal species they belong, have more or less antibody in their sera. We shall soon see what circumstances govern the production of these antibodies and their quantitative variations.

Normal animals injected with suitable repeated doses, either of dead bacilli, or of products derived from the tubercle bacillus and capable of serving as antigens, may likewise furnish sera more or less

[23] Compt. rend. Soc. de biol., 1912, **72**, 15.

rich in antibodies. The horse, the ass, the bullock and the rabbit are the animals of choice for obtaining such sera: the guinea pig should not be used. With L. Massol[24] I made some observations in this regard, from which there stand out certain particularly interesting facts. We did this well before Laub, Sata and Karl Bundschach,[25] who do not even mention our work.

We injected, for example, into a normal horse whose serum contained no trace of antibody, two successive doses of 20 cc. of an extract of tubercle bacilli (containing 2 per cent of dry extract), at 12 days interval. A sudden abundant production of antibodies appeared in the serum of a bleeding on the twelfth day after the second injection.

We continued the injections of bacillary extract in larger doses (40 to 100 cc.), repeated at the same intervals; the antibodies disappeared entirely and no more were formed afterwards.

On the other hand, we injected into another normal horse small doses of bacillary extract (2 cc. diluted in 20 cc. of physiological salt solution) *daily* for 20 days. This animal, beginning the second day after the last injection, furnished a serum which contained much more antibody than that of the horse treated under the preceding conditions.

By way of example, the comparative results of titration of antibody in the sera of the two animals are here given:

DOSE OF COMPLEMENT (FRESH GUINEA-PIG SERUM)	0.5 CC. OF SERUM OF	
	Horse I (massive doses of antigen)	Horse II (divided doses)
cc.		
0.1	−	−
0.2	−	−
0.3	±	−
0.4	+	−
0.5	+	−
0.6	+	±
0.7	+	+
0.8	+	+

The sign − indicates that complete fixation has taken place (no hemolysis).
The sign ± indicates partial fixation; + indicates absence of fixation (hemolysis).

[24] Compt. rend. Soc. de biol., 1910, **68**, 48.
[25] Ztschr. f. Hyg., 1913, **73**, 427.

The serum of horse II is therefore twice as rich in antibody as that of horse I.

We were able to study, in comparison with the serum of our horse II, the antibody content of another serum obtained by H. Vallée (of Alfort) by vaccinating horses with *equine bacilli*, and afterward with *human*.

In each test there were employed 0.05 cc. of horse serum and a guinea pig complement whose minimal activating dose for an hemolytic serum was 0.01 cc.

GUINEA-PIG COMPLEMENT (QUANTITY OF A 1 IN 4 DILUTION)	VALLÉE SERUM	SERUM II
cc.		
0.05	−	−
0.10	−	−
0.15	−	−
0.20	−	−
0.25	±	±
0.30	+	+
0.35	+	+
0.40	+	+
0.50	+	+

The two sera have therefore exactly the same content of amboceptor. Each cubic centimeter fixes $\frac{0.25}{4 \times 0.05} = 1.25$ cc. of fresh guinea pig complement, or 125 minimal activating doses, or 125 units of antibody, according to the system of notation which we have adopted.

In small laboratory animals *not infected with tuberculosis* it is much more difficult to obtain antibodies when the animals are injected with raw tuberculin of Koch, with bacillary extracts (Christian and Rosenblatt, Hamburger and Monti, Laub, Klopstock), or bacillary lipoids (lecithin, cephalin, etc., H. Much, K. Meyer). On the contrary, they readily produce antibody if injected with bacilli killed by heating at 70°C. or even at 100°C. (J. Bordet and Gengou),[26] or again, as did F. Loeffler,[27] with bacilli submitted to prolonged digestion (24 to 48 hours) either with trypsin in alkalin medium, or with the juice of insect-consuming plants of the *Drosera* variety.

[26] Compt. rend. Soc. de biol., 1906, **61**, 218.
[27] Deutsch. med. Wchnschr., 1913, **39**, 1025.

It must be recognized however that the best procedure for enriching sera with antibodies consists in treating, either spontaneously tuberculous animals or those artificially infected, with repeated intravenous injections of virulent tubercle bacilli, or more simply with tuberculins or bacillary extracts subcutaneously. This at any rate is clearly found to be the case with the horse (Vallée, Calmette and Massol), with the bullock (E. Rothe and K. Bierbaum), the goat, the rabbit and the guinea pig (B. Mollers, Klopstock). The same holds true for the tuberculous human being. Clinicians thought that they might take advantage of this fact to follow the effects of tuberculin treatment. Unfortunately it seems to be well demonstrated today that no parallelism exists between the abundance of antibody in the serum and resistance to the disease.

H. PREVENTIVE OR INHIBITORY EFFECT OF CERTAIN SERA OF TUBERCULOUS OR HYPERVACCINATED ANIMALS UPON THE FIXATION REACTION

In an effort to determine the antibody content of a large number of sera of tuberculous persons and hypervaccinated animals, we learned[28] that certain sera possess the curious property of preventing the fixation of complement by antigens and we noted that these same sera may also exercise their preventive or *inhibiting* power in the presence of other sera containing antibodies.

In order to study this preventive or inhibitory property which is above all peculiar to sera of hypervaccinated animals, we made up the following mixture:

20 cc. of serum of hyperimmunized cattle,
1 cc. of an aqueous extract of bacilli, non-peptonated[29] (B^1),
9 cc. of physiological salt solution.

After one hour at 37° and 18 hours in the ice-box a precipitate is formed. The mixture is shaken and divided into 2 equal parts A and B. One of them, A, is centrifuged. The supernatant fluid is decanted and the precipitate suspended in a quantity of saline equal to that poured off.

[28] Compt. rend. Soc. de biol., 1910, **68**, 224; 1911, **71**, 191; 1912, **73**, 120—: Ann. de l'Inst. Pasteur, 1914, **28**, 329.

[29] It should be recalled that this bacillary extract is obtained by macerating 5 gm. of dry bacilli in a liter of water at 65°C. After 48 hours the liquid is filtered and concentrated to 100 cc. This antigen, which we call B^1, contains 2 per cent of dry extract.

The suspended precipitate from A, the liquid decanted from A after centrifugation, and the non-centrifugated portion B are to serve, in different dilutions, for performing the fixation reactions. They are to be employed either alone, or in the presence of a horse serum whose antibody content has been previously determined in the presence of the aqueous bacillary extract B[1].

The experiment shows that all the fixation reactions performed with the precipitate of A, with the liquid decanted from A after centrifugation and with the non-centrifugated portion B, either alone or mixed with 0.5 cc. of horse serum of known antibody strength, are *negative*.

The antigen B[1], combined with hyperimmune cattle serum, has lost the property of fixing complement. *This serum therefore contains an inhibitory substance* (*inhibitrice*) *which prevents* or masks the fixation reaction.

We tried to determine what influence the order of adding the different elements of the fixation reaction might have upon the appearance of the inhibitory substance. The following mixtures were therefore made, each one with amounts varying from 0.1 to 0.6 cc. of fresh complement diluted 1 in 4:

A. 0.5 cc. of antibody-containing horse serum + 0.5 cc. of antigen B[1] diluted to 1 in 40 + 0.5 cc. of physiological salt solution + complement.

B. 0.5 cc. of antigen B[1] diluted to 1 in 40 + 0.5 cc. of inhibitory cattle serum diluted to 1 in 10 + 0.5 cc. of serum antibody + complement.

C. Same test with a *normal* cattle serum in the place of the inhibitory *hyperimmune* cattle serum.

D. 0.5 cc. of antigen B[1] diluted to 1 in 40 + 0.5 cc. of antibody-containing horse serum (30 minutes at 37°) + 0.5 cc. inhibitory cattle serum diluted 1 in 10 + complement.

E. Same test with normal cattle serum replacing the inhibitory hyperimmune cattle serum.

F. 0.5 cc. of antigen B[1] diluted to 1 in 40 + 0.5 cc. of antibody-containing horse serum + complement (1 hour at 37°) + 0.5 cc. of inhibiting cattle serum diluted to 1 in 10 (30 minutes at 37°).

G. The same test with *normal* cattle serum replacing the inhibiting cattle serum.

One should be certain that the inhibiting cattle serum, whether

heated at 58° or not, does not give any hemolysis in the presence of hemolytic serum. This fact is verified by test F above.

In this series of tests, all the tubes of series B and D were hemolyzed. All others gave a positive fixation reaction.

Therefore, *inasmuch as the antigen B^1 and the free antibodies of the horse serum have not fixed the complement, the inhibiting serum prevents the fixation and, after the latter is accomplished, the inhibiting serum cannot liberate the complement.*

Let us now find out exactly the duration of contact necessary if this fixation of complement is to be definite:

In a first test, we determine for differing periods, the fixation of complement by the combination antigen B^1 + horse serum antibody, adding the hemolytic serum and the red cells at the times given in the table.

In a second test, after the same periods of contact of antigen, antibody and complement, we add the inhibiting serum, the hemolytic serum and the red cells.

Finally, in a third test, after the addition of inhibiting serum, at the times indicated, to the combination of antigen + antibody + complement, and incubation for one hour at 37° we add hemolytic serum and the red cells.

Test I

COMPLEMENT 1 IN 4	TIME PERIOD					
	0	5′	10′	20′	60′	
cc.						
0.1	+	+	−	−	−	No inhibiting serum. There is already *clear-cut* fixation after 10 minutes; from then on it increases with time
0.2	+	+	+	−	−	
0.3	+	+	+	+	−	
0.4	+	+	+	+	±	

Test II

COMPLEMENT 1 IN 4	TIME PERIOD				
	0	5′	10′	20′	
cc.					
0.1	+	+	−	−	The addition of inhibiting serum does not liberate fixed complement. Hemolysis corresponds to that produced by the free complement as in Test I
0.2	+	+	+	−	
0.3	+	+	+	+	
0.4	+	+	+	+	

Test III

COMPLEMENT 1 IN 4	TIME PERIOD				
	0	5′	10′	20′	
cc.					
0.1	−	−	−	−	The longer the duration of preliminary contact of complement with antigen and antibody, the less does the inhibiting serum, added after the the complement, prevent fixation.
0.2	−	−	−	−	
0.3	+	−	−	−	
0.4	+	±	−	−	

Once fixation has begun, the inhibiting serum can no longer impede it.

In order that the phenomena of inhibition may be observed with certainty, the inhibiting serum should always be made to act directly upon the antigen before the addition of serum antibody and of complement. The complement should be added last.

We made a comparative study of our sera of hyperimmune cattle and various normal or therapeutic sera (sera of normal cattle, antistreptococcus, antidiphtheria, antitetanus, antivenom, antipest) and we learned that the inhibiting property is met with only in the first. This property is therefore specific. Furthermore, it is not limited to sera of hyperimmunized cattle, inasmuch as we found it also in certain sera of the horses vaccinated by Vallée (of Alfort) and Rappin (of Nantes). It also exists in certain other animals and has been met with at times, though exceptionally, in tuberculous man (Calmette and Massol, Caulfeild and Beatty).[30]

We were able to prove that the inhibiting power of serum of hyperimmune cattle increases immediately following intravenous injections of bacilli. It reaches its maximum after 5 to 6 days, then falls, without however disappearing entirely, even though the animal be left undisturbed for two months.

Cattle recognized as tuberculous at the abattoir usually do not have an inhibiting serum. We found only one such in which the activity was evident. This animal, which had very extensive chronic lesions, was moreover in apparently excellent condition.

The serum of small laboratory animals (guinea pigs and rabbits) infected subcutaneously does not possess inhibitory properties.

Our tests have shown that the inhibiting substance acts upon tuberculous antigens, of whatever nature, including the bacilli. It

[30] J. Med. Research., 1911, **24**, 101.

masks antibodies contained in the sera of the same species as well as those contained in the sera of foreign species. Its action is the more intense the more suitable is the dose of inhibiting serum employed.

The inhibiting sera which we have studied do not give the fixation reaction in the presence of our aqueous bacillary extract antigen (B^1).

But if we use as antigens either killed and washed bacillary bodies (in a dilution of 0.5 per cent of dry bacilli), or peptonated bacillary extract (antigen B^2), a dose of 0.2 cc. of these antigens enables us always to detect antibodies and to obtain a positive reaction with an appropriate quantity of serum.

It is seen therefore that the inhibiting sera themselves contain antibodies which are thus unmasked and which may be measured accurately by varying, on the one hand the doses of antigen employed, and on the other, the quantities of sera used.

I. NATURE AND FUNCTIONS OF THE INHIBITORY SUBSTANCE (INHIBITRICE)

It is known that the sera of certain tuberculous persons and that of hyperimmune animals give a precipitate in contact with tuberculin (Calmette and L. Massol).[31] This fact brought up the question as to whether the reaction of precipitation intervened in some way in the phenomenon of inhibition.

In order to separate the tuberculin precipitable substances from the inhibiting serum, it is sufficient to make a mixture in the proportion of 1 cc. of serum to 0.125 cc. of aqueous bacillary extract B^1. The two are left together for 2 hours in the incubator and 18 hours in the ice-box, and then centrifugated in order to separate the precipitate. If now, more bacillary extract is added to the supernatant fluid, there is no longer any clouding. Yet the supernatant fluid retains practically the half of its inhibiting properties.

A still more clear-cut result is obtained by treating the inhibiting serum with a non-antigenic tuberculin (precipitated with alcohol), or again with mallein which likewise gives a precipitate. The fluid, on being rid of the precipitate, preserves its inhibitory power almost completely.

It must therefore be granted that the precipitation of serum by antigen does not play any rôle in the reaction of inhibition.

[31] Compt. rend. Acad. des sci., 1909, **149**, 760; 1910, **151**, 285.

We should recall too that the so-called antituberculous serum of Ruppel and Rickmann, for example, very rich in antibodies, gives an abundant precipitate with various tuberculins and, at the same time, possesses no inhibiting power.

Other experiments have indicated to us that an unheated inhibiting serum, freed of complement by aging, loses about 33 per cent of its inhibitory substance on being heated to 58° for three hours. This same serum, on being heated and then mixed with bacillary extract, does not furnish more than 31 per cent of the amount of precipitate which was to be separated off by centrifugating when it was mixed with the antigen, without having been heated.

Precipitation with distilled water (with subsequent adjustment of tonicity) gives a less definite result. With the bacillary extract, there is obtained not more than 42 per cent of the precipitate given by the normal serum and 50 per cent of the inhibitory substance contained in the non-treated serum.

A part of the inhibitory substance is therefore carried down with the precipitate produced in an inhibiting serum.

It might still be asked whether the reaction of inhibition is not the result of the presence in the serum of an excess of antibody.

Now, the serum of Ruppel and Rickmann, which is richer in antibodies than any we have ever had, is never inhibiting, no matter what the quantity used in the presence of a like amount of antigen. And on the other hand it is found, as we have already said, that inhibiting sera do not liberate the antibodies which they contain along with the inhibitory substance, no matter how small the dose in which they are employed, except when they are combined with a large dose (about 3 times larger than that required by the serum of Ruppel and Rickmann) of appropriate antigen, such as our peptonated antigen B^2. The fixation of complement therefore cannot take place through the action of antibodies upon antigen except when all the inhibiting substance has been saturated by this antigen.

The combined action of dilution with distilled water and of a current of carbon dioxide (*method of* Liefmann) upon an inhibiting serum may make it possible to separate the inhibitory substance from the antibodies (Calmette and Massol).[32]

Into an inhibiting cattle serum inactivated at 58° and diluted with 9 volumes of distilled water, we pass a current of carbon dioxide.

[32] Compt. rend. Soc. de biol., 1913, **75**, 160.

After standing for two hours and being centrifugated, the liquid is decanted and rendered isotonic. The abundant precipitate formed is taken up with physiological salt solution.

The following are the weights of dry precipitate furnished by 1 cc. each of various sera.

	grams
Inhibiting cattle serum	0.0225
Serum of *normal cattle*	0.00875
Ruppel and *Rickmann* serum	0.0125
Antivenom serum	0.008
Horse serum agglutinating the typhoid bacillus	0.009
Normal human serum	0.005
Normal guinea pig serum	0.00375

The inhibiting cattle serum therefore yields a much more abundant precipitate than the serum of normal cattle.

In other experiments we studied the inhibiting power of the liquid, of the precipitate and of the mixture reconstituting the original serum, and we compared them with the inhibiting power of the latter. Thus it was found that the decanted liquid does not possess inhibiting power, but the precipitate, on the contrary, shows itself to be as active as the serum from which it was derived. The precipitate from the serum of Ruppel and Rickmann, or from healthy cattle serum does not hinder the fixation. *The reaction is therefore specific.*

The search for antibodies in the various fractions of the serum gives a negative result with antigen B^1. With antigen B^2 (peptonated extract of bacilli) it is found that the precipitate retains small traces of antibody; the decanted liquid on the contrary, even when employed in large excess, gives a very clear-cut fixation which is not attenuated. It is therefore non-inhibiting. In order to bring about a reappearance of the inhibition, one needs only to incorporate the precipitate into the decanted liquid.

Furthermore, if a fixation reaction is performed with antigen B^2 and some decanted liquid, or with the original serum, it is observed that, in order to obtain an equal degree of deviation with the decanted liquid, about three times less antigen is required than with the serum (a distinctive characteristic of non-inhibiting antibody sera and inhibiting sera).

It should be added that the precipitins are recovered with the inhibitory substance in the precipitate, while the agglutinins remain

with the antibodies in the decanted liquid. The inhibitory substance is therefore quite distinct from the antibodies. The latter are also, as a result, distinct from the precipitins (*see Chapter XXX, F*).

How are we to explain the formation of this inhibitory substance in the serum of hyperimmune animals and its customary absence in the blood of tuberculous animals?

We can only formulate an hypothesis: namely, that in animals immunized by massive intravenous injections of more or less modified or virulent tubercle bacilli, the latter, immediately sensitized by an overabundance of antibody, induce an enrichment of the blood in globulins (as suggested moreover by the fact that the serum, even heated at 58°, forms an abundant precipitate of globulins if carbon dioxide is bubbled through it (method of Liefmann)). Now these globulins, which contain the inhibitory substance, as we have demonstrated, have the property of preventing the fixation of complement to the sensitized antigens and even of impeding agglutination.

We have seen earlier (*Chapter XXXV, C*) that, according to the studies of Friedberger and Goldschmidt,[33] G. Shibayama, and others, tubercle bacilli combined *in vitro* with fresh guinea pig serum may, after centrifugation, give rise to a so-called *anaphylatoxic* reaction on intravenous injection into a normal guinea pig.

F. Neufeld and H. Dold[34] arrived at the same result by intravenously injecting a series of guinea pigs, of about 200 gms. weight, with a few milligrams of human or bovine bacilli. For one portion of the animals (the others remaining as controls) the bacilli had been sensitized by 24 hours of contact in the ice-box or incubator with a specific serum (serum of goats immunized by the authors or serum of Ruppel and Rickmann prepared by Hoechst), and then combined with fresh guinea pig serum for 24 hours. In 9 cases they thought that they were able to isolate thus, from the treated bacilli, a toxin killing the guinea pig in 2 to 5 minutes. All the positive results were obtained with bacilli sensitized in the ice-box and macerated afterward in the incubator in the presence of fresh complement. The bacilli sensitized simply by specific serum never gave an anaphylatoxic reaction.

On the hypothesis that the intravenous injection of bacilli into animals sensitized by previous injections produces an *anaphyla-*

[33] Ztschr. f. Immunitätsforsch., 1911, **9**, 369.
[34] Arb. a. d. k. Gsndhtsamte, 1912, **38**, 275.

intoxication (death has occurred in the course of immunization by intravenous injection, avoidable oftentimes by Besredka's procedure of subintrant vaccination), one might think that the inhibitory substance (inhibitrice), which opposes itself to the fixation of complement, conspires thus to prevent the anaphylatoxic reaction which is associated with the lowering of the complement strength (J. Bordet, Muttermilch). It is well to remark that we have not been able to corroborate this fact by direct experiment, since the inhibiting cattle serum at our disposal is toxic for the guinea pig in a dose at which inhibition is insufficiently marked. We believe however that the inhibitory substances exert in the body a protective or defensive action against intravenous injections and massive bacillary infections. This opinion seems justified by the fact that if the injection of bacilli into hyperimmune animals is discontinued, a diminution in the inhibiting power of their sera results.

K. EXAMINATION FOR ANTIBODIES IN EXTRACTS OR ORGANS AND IN TUBERCULOUS EXUDATES

Just as the antitoxins are elaborated principally by the leucocytes, as brought out by the work of Metchnikoff and his pupils, it seems indeed that the cells and organs which are chiefly concerned in leucocyte production, such as the lymphatic glands, the bone marrow and the spleen are the principal organs of elaboration of tuberculous antibodies. One would expect then to find them in these organs in abundance, and the fixation reaction of Bordet-Gengou makes it possible to demonstrate that this is the case.

Livierato[35] detected them readily in scrofulous and tuberculous glands by using suspensions of bacilli, bacillary extracts or tuberculin as antigens, and P. Paraskcropoulos[36] showed that they are found in larger quantity in the sero-fibrinous exudates of acute pleurisies than in the serum of the patients themselves, if the bacilli contained in these exudates are first carefully eliminated by centrifugation.

In conclusion, L. Karwacki and Czeslas Otto,[37] studying the sputa of cases of pulmonary tuberculosis, have likewise been able to demonstrate the presence of antibodies, even when these sputa already contained bacilli. The antibodies were therefore present in excess.

[35] Centralbl. f. Bakt., 1911, **57**, 366.

[36] Compt. rend. Soc. de biol., 1911, **70**, 586.

[37] Ibid., 1911, **71**, 523.

L. HEREDITARY TRANSMISSION OF TUBERCULOUS ANTIBODIES

It is known from the work of Theobald Smith, Rosenau and J. F. Anderson, that young guinea pigs born of mothers sensitized to horse serum, retain for several weeks after birth a certain degree of hypersensitiveness to this same serum. It may be pertinent to ask therefore whether, in tuberculous infection, the antibodies pass from mother to fetus. This question has been studied by several investigators.

J. Parisot and Hanns[38] autopsied a pregnant woman who died of tuberculosis with cavities during the eighth month of gestation. After the death of the mother the heart beat of the infant was still perceptible and it was removed by cesarean section, but survived only a half hour. The maternal and fetal placenta, the liver, the spleen and suprarenal capsules were collected aseptically. Their histological examination disclosed neither tubercles nor tubercle bacilli. Guinea pig inoculation remained negative. But the reaction of Bordet-Gengou was positive both with the blood of the mother and with that of the infant.

Experimentally, Schenck[39] had obtained an identical result with the blood of two young guinea pigs born of a mother tuberculized, and later treated with increasing injections of bacilli. On the other hand Fedeli[40] was completely unsuccessful in his search for antibodies in the blood of the young female guinea pigs merely infected and not treated.

Esther Rosenkrantz[41] studied in my laboratory the sera of 100 infants, almost all of them born at the Baudelocque Maternity at Paris in the service of Professor Pinard. She could not determine as to whether the mothers were tuberculous and reacted to tuberculin, but of these 100 new born, 31 had tuberculous antibodies which could be detected by the reaction of Bordet-Gengou, using as antigen a suspension of bovine bacilli killed by heating at 100°. The blood was simply taken from the umbilical cord at the time of birth.

This proportion of 31 per cent of infants showing antibody corresponds approximately to what we know of the frequency of latent

[38] Rev. méd. de l'Est. 1910, April 15.
[39] München. med. Wchnschr., 1910, **57**, 2514.
[40] Ztschr. f. Immunitätsforsch., 1911, **9**, 1052.
[41] Compt. rend. Soc. de biol., 1911, **71**, 142.

tuberculous infection in mothers of healthy appearance. Now persons who harbor latent lesions often have antibodies in their serum. It seems therefore indeed that the passage of tuberculous antibodies from mother to fetus should be regarded as the rule, at least in the human race. But it is desirable that numerous and more accurate studies be made to throw light upon this question, since it is possible,—and certain facts observed by Boez in my laboratory support this supposition,—that the serum of new born infants and that of pregnant women, as well as certain sera of syphilitic subjects, contain substances capable of fixing complement in the presence of tuberculous antigens.

M. THE DIAGNOSTIC AND PROGNOSTIC IMPORTANCE OF THE DETECTION AND TITRATION OF ANTIBODIES IN TUBERCULOUS INFECTION

When the sera of normal persons, of individuals suspected of tuberculosis or patients clinically tuberculous, are methodically examined for antibodies, the latter are found to be always lacking in normal persons while almost always present in those who have progressive tuberculosis.

This study was made first by F. Widal and Lesourd, Camus and Pagniez in 1901, then by Wassermann and Bruck, Calmette, L. Massol and M. Breton,[42] L. Michaelis and Georg Eisner,[43] Marmorek, Bergeron,[44] Otto Deilmann,[45] and others; but since different techniques, often defective, were employed their results admit of no comparison.

Thus Wassermann and Bruck found antibodies only in the serum of tuberculous cases treated with tuberculin, while it is today demonstrated that in the absence of any treatment these antibodies exist in about 80 per cent of patients. Their finding is correct only to the extent that injections of tuberculin in general augment the antibody content of the serum. This fact has been brought out by our experiments.[46]

We treated a series of 8 patients for five months with fractional

[42] Compt. rend. Soc. de biol., 1908, **65**, 648.
[43] Ztschr. f. Immunitätsforsch., 1910, **6**, 571.
[44] Compt. rend. Soc. de biol., 1911, **70**, 176.
[45] Ztschr. f. Immunitätsforsch., 1911, **10**, 421.
[46] Compt. rend. Soc. de biol., 1912, **73**, 122.

and progressive doses of aqueous bacillary extract, in such a way that they received a maximal dose of 833 antigen units (corresponding to 4.1 milligrams of dry extract). The progression of antibody content before and after the treatment was the following for each of them:

PATIENT'S NUMBER	STAGE ACCORDING TO TURBAN	UNITS OF ANTIBODY WITH THE PEPTONATED EXTRACT B^2	
		Before treatment	After treatment
14	1st	5	333
15	3rd	15	200
16	3rd	33	500
17	1st	15	100
18	2nd	100	333
19	1st	5	333
20	3rd	5	100
21	3rd	0	100

Of 134 sera of non-treated patients, divided into 3 groups according to the extent of their pulmonary lesions and following the classification of Turban, in 127 we found antibodies capable of detection by our peptonated antigen B^2. They were grouped as follows:

DEGREE ACCORDING TO TURBAN	NUMBER OF PATIENTS	FIXATION REACTION		PERCENTAGE OF POSITIVE REACTIONS
		Positive	Negative	
				per cent
I	27	26	1	96.29
II	46	43	3	93.4
III	61	58	3	95.0

In this series therefore the proportion of positive reactions reaches 95 per cent. Other workers who used our peptonated antigen B^2 obtained analogous figures (Armand-Delille, and Rist and Vaucher,[47] for example).

With other antigens the results are somewhat different. Nevertheless A. Besredka, F. Jupille, Debains and Manoukhine,[48] and J. Bronfenbrenner[49] with egg culture centrifugated and sterilized at

[47] Compt. rend. Soc. de biol., 1913, **74**, 791.
[48] Ibid., 1914, **76**, 180; 199.
[49] Ztschr. f. Immunitätsforsch., 1914, **23**, 221.

120°C., find a constantly positive reaction in 100 cases of pulmonary tuberculosis: negative in only 11 phthisical cases in the third stage and negative also in 43 non-tuberculous persons ill with other diseases.

Kuss, Leredde and Rubinstein,[50] using the same egg culture of Besredka, report 89 per cent of positive reactions in subjects affected with actively progressive pulmonary tuberculosis and 66 per cent in apyretic tuberculous cases in the early stage.

With the raw tuberculin of Koch as antigen, Wolff and Hans Mühsam[51] found only 46 positive reactions and 32 doubtful reactions among 109 patients in various stages. Sig. Cohn under the same conditions found only 15 among 53 patients in the second and third stages. F. Bezançon and de Serbonnes,[52] using either raw tuberculin, or ground up human bacilli as antigens, were able to find antibodies in only one-third of their cases.

In using a suspension of human bacilli cultured upon potato, Elisabeth T. Fraser[53] obtained 8 positive reactions among 13 sera of tuberculous patients, that is 61 per cent, and 8 negative results among 8 sera of non-tuberculous subjects.

At the Trudeau laboratory at Saranac Lake, H. M. Kinghorn and D. C. Twitchell[54] used suspensions of human bacilli and reported 13 positive results among 14 advanced tuberculous cases, that is 93.3 per cent, only 3 positive results among 8 incipient cases, or 37.5 per cent and 7 negative results among 7 normal subjects.

With an antigen prepared by S. A. Petroff, also at Saranac Lake (extract of 1 gm. of tubercle bacilli dried in vacuo over sulphuric acid, triturated in a glass ball grinder with 100 gms. of water containing 25 per cent of glycerin, then gently heated at 105°C. for one hour and decanted), Lawrason Brown and S. A. Petroff[55] found 72 per cent of positive fixation reactions among 478 patients ill with pulmonary tuberculosis.

A fairly large number of the cases who react to a subcutaneous injection of tuberculin have no antibodies in their sera.

The antibody content of the serum of patients who have not

[50] Compt. rend. Soc. de biol., 1914, **76**, 244:—Bull. méd., 1914, **28**, 622.

[51] Deutsch. med. Wchnschr., 1908, **34**, 1504.

[52] Compt. rend. Soc. de biol., 1909, **67**, 548.

[53] Ztschr. f. Immunitätsforsch., 1913, **20**, 291.

[54] Ztschr. f. Tuberk., 1913, **20**, 11.

[55] 34th Ann. Rep. Trudeau Sanatorium, Saranac Lake, 1919.

received tuberculin is usually small. According to our titrations it seldom exceeds 50 units in the tuberculous in the second and third stages; 20 units only in the first stage.

Our figures show the amount of antibody to be nil or little at the beginning of the infection. It increases as the disease progresses, and the antibodies disappear totally at the end when the cachexia becomes established and death is near.

At the Emile Roux dispensary, at Lille, Boez, using our antigen B^2, studied the sera of a fairly large number of patients in different stages of their disease. He finds among pulmonary cases:

77.7 per cent of positive reactions in the first stage;
82.0 per cent of positive reactions in the second stage;
63.6 per cent of positive reactions in the third stage.

He notes, on the other hand, 32.5 per cent of positive reactions in so-called pre-tuberculous cases who present either the signs of Grancher or suspicious glandular enlargements; 18.8 per cent of positive reactions in subjects ill with various non-tuberculous affections, and nothing but negative reactions among 7 healthy individuals whom he was able to follow and who had all given him a positive tuberculin cuti-reaction.

By titrating the antibodies found in his patients, Boez has demonstrated moreover that no evident relation exists between the form of evolution and the antibody content of the serum. He has also observed, as had Wolff and Mühsam,[56] and contrary to the opinion of Armand-Delille,[57] that no parallelism can be detected between the antibody content and the intensity of the cuti-reaction. Patients ill with surgical tuberculosis for example, although they show no sign of glandular or pulmonary tuberculosis, react very violently to tuberculin, and yet their blood contains no trace of antibody. The same is true of old healed cases of tuberculosis (Caulfeild and Beatty)[58] and of cases of lupus (Capelli).

It may be said that, in a general way, almost all authors who have sought tuberculous antibodies with a satisfactory technique, arrive at the conclusion that the elaboration of antibodies by the body indicates always an evolutive form of the disease. Their presence is therefore of real diagnostic value. They are more abundant in the

[56] Deutsch. med. Wchnschr., 1908, **34**, 1504.
[57] Compt. rend. Soc. de biol., 1909, **66**, 706.
[58] J. Med. Research, 1911, **24**, 101.

more advanced stages than at the beginning, so that, as regards the prognosis, periodical titration is of great interest. Finally, their total disappearance, coinciding with an aggravation of clinical signs, presages cachexia and early death.

N. THE RÔLE OF ANTIBODIES IN THE DEFENSE OF THE BODY AGAINST TUBERCULOUS INFECTION

Wassermann and Bruck,[59] and Citrons,[60] have advanced the hypothesis that the tuberculin reaction is due to the fact that tuberculin unites itself to antibody formed in the tuberculous foci. In order to react against the toxic products elaborated by the bacilli, the cells are said to give off receptors which combine with these substances to neutralize them. Under the influence of the cellular stimulation there should be an over-production of receptors at the same time that there is an over-production of free antibody.

When a small quantity of tuberculin is injected, the normal cells are said not to be influenced, but the cells of the tuberculous foci should react to combine their receptors with the tuberculin. This cellular reaction is expressed by the febrile reaction (*see Chapter XXXV C*).

The conception of Wassermann and Bruck tends therefore to identify the antibodies with an antituberculin whose function consists in neutralizing the tuberculin produced in the midst even of tuberculous lesions or introduced artificially into the body. According to Lowenstein[61] one should be able to demonstrate the existence of this antituberculin by mixing a constant quantity of tuberculin with varying amounts of the sera of tuberculous cases, and by using these mixtures (left in contact beforehand, first for 2 hours in an incubator, then 20 hours in an ice-box) for cuti-reactions upon the arm of a patient, a tuberculin control test being made at the same time. Thus one should find that a certain proportion of sera inhibit the cuti-reaction judged, in this case, by the appearance of the characteristic papule.

Our experiments (Calmette and L. Massol)[62] have convinced us that, as a matter of fact, it is not at all a true neutralization. We

[59] Deutsch. med. Wchnschr., 1906, **32**, 448.
[60] Berl. klin. Wchnschr., 1907, **44**, 1135.
[61] Ztschr. f. Tuberk., 1909/10, **15**, 337; 458.
[62] Compt. rend. Acad. des sci., 1910, **151**, 285; 1911, **153**, 420.

shall dwell further upon the interpretation of their results (*Chapters XLI and XLII*). Prolonged contact of serum with tuberculin leads simply to a precipitation of serum globulins, and if cuti-reactions are performed with the precipitate freed from the serum by centrifugation, and with the serum separately, it is seen that the precipitate gives neither the cuti- nor the ophthalmic reaction, whereas these reactions are produced by the serum which contains the whole of the initial tuberculin. The tuberculin is therefore not modified either in its nature or in its effects; it is not *sensitized* as Lowenstein,[63] Pickert,[64] H. Vallée,[65] Finzi and other authors believed.

It is easy to prove that sera which are richest in antibodies have no *neutralizing property in vitro* for tuberculins, if the effects of mixing them are gauged by the method of intracerebral inoculation into the tuberculous guinea pig. Besides, Lowenstein himself recognized that antibodies demonstrable by the Bordet-Gengou test are often found in sera which show themselves incapable of neutralizing the cuti-reaction effects of the tuberculin.

I have already said that in the present state of our knowledge, it does not seem that there can be established any strict correlation between the appearance of antibodies in the serum of a given individual and the aptitude of the latter to react to tuberculin, inasmuch as cases with bone or joint lesions, lupus, and apparently cured glandular tuberculosis usually react very violently to this substance, while the examination for antibodies in them is almost constantly negative.

If the local or general tuberculin reaction followed upon the union of tuberculin with an antituberculin, in the sense of Wassermann and Bruck, it would be incomprehensible that antibodies cannot be detected in precisely those individuals in whom this reaction is the most intense.

Inversely moreover, in certain animals artificially infected, it has been possible to observe the appearance of antibodies before the capacity to react to tuberculin. For instance Slatineanu, Danielopolu[66] and Besredka and Manoukhine[67] found them after the 4th day

[63] Deutsch. med. Wchnschr., 1908, **34**, 2262.
[64] Ibid., 1909, **35**, 1013; 1514.
[65] Compt. rend. Soc. de biol., 1909, **67**, 700.
[66] Ibid., 1909, **66**, 61.
[67] Ibid., 1914, **76**, 180: —Ann. de l'Inst. Pasteur, 1914, **28**, 569.

in the guinea pig. This interval is however extremely variable and experimentation up to the present has not justified us in laying down any precise rules in respect to it. The only fact which does stand out clearly is the effect of repeated injections of tuberculin, of bacillary extracts, of dead bacilli or of small doses of virulent or attenuated living bacilli, upon the increase of antibody content in the serum, even when the injections are unfavorably influencing the evolution of the lesions.

Antibodies cannot therefore be regarded as the essential elements in the defense against tuberculous infection. They appear to be rather the *evidence* of cellular reactions against the toxic products excreted by the bacilli in infected tissues, or against the tuberculin introduced artificially into the normal or diseased body. But they do not *neutralize* the tuberculin. There is no parallelism between their presence in the serum of an individual and his capacity to react to tuberculin. And if it is demonstrated that their disappearance, in subjects gravely infected, is of very serious import, it seems well proved that their relative abundance in no way indicates a state of immunity or resistance to infection.

PART FOUR

Natural Immunity and Processes of Immunization Against Tuberculous Infection

CHAPTER XXXVIII

NATURAL VARIATIONS IN THE VIRULENCE OF THE TUBERCLE BACILLUS

If cultures of tubercle bacilli from various sources are studied at the moment of their isolation and before successive passages and long cultivation upon artificial media have modified their characters, it is easy to demonstrate that the virulence of the well defined types, —*human, bovine, avian,*—is remarkably constant. Like weights of cultures of the same age of human or bovine bacilli, from different subjects, almost always give rise in the guinea pig—the animal reagent par excellence—to a tuberculosis which evolves in the same manner, provided the conditions of inoculation are identical.

The severity or mildness of tuberculous infections for this or that susceptible animal species evidently depends, as I have already shown by experiments cited in Chapter XX (A), upon two principal factors: one, the *quantity of bacilli absorbed* at a single time, or on several occasions at intervals so close that the defensive cellular reactions have not been able to act; the other, the *localization of the primary infection,* that is to say the place in the body where the first follicular lesion forms.

This is certainly the case with the human race, inasmuch as there is no reason for believing that man responds differently to tuberculous virus than animals which, like himself, are subject to spontaneous infection.

Among the latter, cattle, for example, without regard to race, show themselves prone to contract tuberculosis when placed under the same living conditions and exposed to the same factors of contamination. We know indeed that tuberculous infection is more prevalent among the herds of some regions than of others, and veterinarians have thought that they noticed a greater susceptibility among certain breeds, for example in the cattle of the "race lourdaise" in the south of France, or the Durham breed in England. But on studying the question more closely, they became convinced that it is in reality the manner of raising as well as technical methods of

selection and crossing which have increased the risk and chances of infection. Permanent or prolonged confinement in stables, and the introduction of breeding animals from infected herds into tuberculosis-free herds, are alone responsible for the diffusion of tuberculosis among the cattle of all countries.

As a matter of fact, there is no race of cattle which is particularly susceptible, nor is there any race particularly refractory. And if it is true that cattle of the race of Lourdes or those of Charolais-Nivernais are proportionally more infected,—all other conditions being equal—it it due to the fact that in these races, in which the tendency to albinism is so remarkable, the more highly developed lymphatic system offers a more extensive surface for absorption of infectious elements of all sorts.

The same applies in man to those persons to whom Landouzy applied the term *vir rufus*, individuals with golden or red silky hair, with pale, fine, transparent skin, and with a freckling which recalls the beauties of the Venetian school of painting.

For all men, as for all cattle, no matter what their race, *when they afford a virgin soil free from all infection or preëxisting impregnation of bacilli*, the natural tuberculous virus,—that is to say, that derived from tuberculous lesions and not from cultures,—is never *innocuous*. The intensity and the severity of the lesions which it produces are in proportion to the *quantity* of bacilli introduced into the body, much more than to their *quality*, the variations of which, in so far as concerns the human or bovine virus taken separately and recently from the tuberculous organism, are of very little importance.

Attempts on the part of various workers to find whether differences in virulence exist between cultures of tubercle bacilli isolated from very diverse human lesions have, up to the present time, given results which are almost entirely negative. Krompecher and Zimmermann,[1] for example, sought in vain for differences in strains of bacilli from surgical tuberculoses, and C. Fraenkel and E. Baumann[2] with 37 strains isolated from lung cavities, as well as from the pus of white swellings, were never able to find a race of human bacilli which showed itself, for the guinea pig, devoid of or even attenuated in virulence.

[1] Centralbl. f. Bakt., 1903, **33**, 580.

[2] Ztschr. f. Hyg., 1906, **54**, 247.

Other attempts by Moeller were no more successful.[3] The *English Commission* however called attention to the lessened virulence (for the calf, rabbit, guinea pig and monkey) of 7 cultures of the bovine type and of 4 of the human type isolated from cases of lupus in man. Some analogous facts have been pointed out by other workers, but the experimental criteria for determining the differences of activity of the virus are deficient. For the results to be comparable, all experiments should have been carried out upon animals of the same species, the guinea pig for example, with cultures derived either directly from human or bovine lesions, or from the first or second passage through the guinea pig (the body of the animal not appreciably modifying the original characters of the human or bovine virus, at least under these conditions). Furthermore the same test technique should have been applied uniformly, for example, as done by Et. Burnet.[4] Each culture strain should have been inoculated into a series of guinea pigs of the same weight, under the skin of the thigh, in doses of 0.25, 0.01, and 0.001 mgm. of bacilli (weighed fresh).

In this way Et. Burnet learned that neither tumefaction of different gland groups, nor of the spleen, gives any measure of virulence. It is only by the degree of involvement of the lungs, being the last organs *visibly invaded* after these subcutaneous inoculations, that one may judge as to the rapidity of the extension of the tuberculous infection. Also it is well to sacrifice at the end of 8 weeks a part of the animals inoculated with 0.25 mgm., since if lesions are not apparent by then with this strong dose of virus, it is because the latter is greatly attenuated.

In a first series of experiments, upon 42 strains studied under these conditions and derived from children and from adults, from pulmonary, gland, bone and cutaneous tuberculoses, Burnet could not find a single one which could be regarded as *attenuated*. He even found that, in the majority of cases, the virulence of bacilli isolated from external tuberculoses was greater than that of bacilli from sputum taken as controls. "Far from bone and joint tuberculoses of children being caused by attenuated bacilli," says he, "the facts go to prove that these bacilli are fully virulent, either because the young body offers poor resistance, or because their source lies in an

[3] Ztschr. f. Hyg., 1906, **55**, 506.

[4] Ann. de l'Inst. Pasteur, 1912, **26**, 868; 1915, **29**, 221.

almost direct contagion from active tuberculoses, or because they are endowed with a special aptitude for mobilizing and scattering themselves by the lymph and the blood in the infected organism."

Yet among 14 strains of bacilli isolated by guinea pig inoculation from cutaneous and spontaneous tuberculoses, 4 appeared definitely attenuated and were all of the human type. This was notably the case in an individual who from childhood had suffered with a torpid cutaneous tuberculosis of the leg, from which Burnet succeeded in isolating, after two successive passages through the guinea pig, a bacillus which showed itself very benign. Whereas the other bacilli killed monkeys (*macacus and cynocephalus*) within 70 days at the longest on subcutaneous inoculation of 1 millionth of a milligram or on two ingestions of 1 and 2 mgms., this benign bacillus, in the same doses, permitted them to live considerably longer.

The question may be asked therefore whether, after several passages through an animal body like that of a guinea pig or monkey, the bacilli of attenuated virulence, like the one just described, are not susceptible of recovering their initial virulence. That is what happened in fact to Burnet's bacillus. The latter on being recovered from a lymph node of a monkey in which it had lived for three months, showed itself distinctly virulent for the guinea pig, since a dose of 0.25 mgm., inoculated subcutaneously, produced in 9 weeks a generalized tuberculosis with pulmonary tubercles. On the other hand, Burnet has noted that a bacillus attenuated for the guinea pig does not acquire a greater virulence by several passages through guinea pigs, and that the tuberculin prepared from several strains of attenuated bacilli has shown itself just as active as the tuberculin from a very virulent bovine bacillus.

The *English Commission* concluded from its experiments that *attenuated* bovine and human bacilli, on being passed several times through the calf, guinea pig or monkey, regained a virulence equal to that which characterizes the bovine bacillus for cattle, and the human bacillus for the guinea pig or monkey. The "attenuations" observed in experimenting with certain cultures are therefore only *apparent* and *temporary*, they result from environmental influences, as is the case with simple aging in artificial cultures. They are not *definitely acquired.*

In the present state of our knowledge therefore, it does not seem that we can think of utilizing any one of these bacilli of temporarily

obscured virulence as a vaccine for man or cattle, under the conditions in which we employ cowpox against variola, for example. It seems on the contrary that such bacilli should be regarded as capable of propagating tuberculosis. Individuals and animals which give them shelter in their bodies, without themselves suffering, may eliminate and cast them off by the normal avenues (bile and excrements) as I have demonstrated with C. Guérin. They are carriers and *intermittent disseminators of bacilli*, and are all the more dangerous in that they arouse no suspicion in those about them.

CHAPTER XXXIX

NATURAL IMMUNITY.—"PHENOMENON OF KOCH" AND RESISTANCE OF TUBERCULOUS INDIVIDUALS TO FURTHER INFECTIONS BY THE BACILLUS

A. NATURAL IMMUNITY

When tubercle bacilli of human or bovine origin are inoculated subcutaneously into birds, or even into certain mammals but slightly susceptible to tuberculous infection, such as the *gerbilles* (*Meriones shavii*) or the *spermophiles* of the Russian steppes (*Spermophilus citellus* and *fulvus*), it is found that the bacilli do not invade one lymphatic gland after another as in the more susceptible animals, and that the lesions which they determine, although presenting usually the characteristic appearance of the tuberculous nodule, do not become generalized; they remain *local* and do not lead to any serious disturbances of function. The injected bacilli stay in the immediate neighborhood of the site of inoculation, enclosed in the macrophage cells, and generally do not multiply. They alter themselves more or less in the course of time and finally lose their vitality and even their form (Metchnikoff); but during a very long period, sometimes for several years, they remain alive and are capable of manifesting their virulence if inoculated into a guinea pig, for example, after being liberated from the mass of connective tissue surrounding them. And yet the animal which was harboring them did not suffer at all: it was presenting therefore a *natural immunity* to tuberculous infection.

The mechanism of this immunity has been studied by Dembinski,[1] in Metchnikoff's laboratory at the Pasteur Institute. This scientist proved that the bacilli of human tuberculosis for example, when introduced into the body of a pigeon, collect in masses which are soon imprisoned by true giant cells or polynuclear macrophages. The leucocyte microphages in this case do not play any effective rôle. The macrophages are incapable of destroying the bacilli, but they wall them in and prevent them from multiplying.

[1] Ann. de l'Inst. Pasteur, 1899, **13**, 426.

The tubercle bacilli of warm-blooded animals, introduced into the organism of cold-blooded animals,—let us say into the dorsal lymph sac of the frog,—behave in the same manner. They are to be recovered after several weeks, often after several months, ingested into the leucocytes, but still intact, alive and reinoculable into a susceptible animal.

These non-tuberculizable animals are therefore *refractory* by nature: the bacilli remain in their body fluids or in their tissues *like harmless foreign bodies.* They *tolerate them without being able to destroy them* but a symbiosis is never established between the bacilli and the cells which take them in.

It is a tolerance of this sort which artificial immunization should aim to produce. It would be vain in fact to hope to confer upon the organism susceptible to tuberculous infection the faculty of digesting tubercle bacilli, when refractory animals do not succeed in this! The membrane formed of chitin, of waxes and of fats, which envelops the toxic and toxigenic protoplasm of the bacilli, constitutes so great an obstacle to the digestive action of the leucocytes that one cannot conceive of immunity to tuberculosis as the result of a process analogous to that which intervenes in immunity to acute infectious diseases,—a process characterized by rapid and massive formation and liberation of antitoxins and bacteriolysins into the body fluids.

This idea stands out distinctly from all the experimental work undertaken up to now in the various laboratories and which has not yet succeeded in discovering a method of vaccination capable of being applied to the prevention of tuberculosis in the human race. But the enormous amount of work done and the various attempts inspired by it have given us fortunately a mass of facts and observations of which it is most important for us to make use quickly and to advantage.

B. PHENOMENON OF KOCH.—RESISTANCE TO SUPERINFECTION

The point of departure of the really fruitful researches of late years upon immunity to tuberculosis, lay in the curious finding on the part of R. Koch in 1891[2] and which he described in the following manner:

"If a healthy guinea pig be inoculated with a pure culture of bacilli, the wound ordinarily closes and appears to heal from the

[2] Deutsch. med. Wchnschr., 1891, **17**, 101.

beginning. But toward the tenth or fifteenth day there appears, at the point of inoculation, a hard nodule which soon opens spontaneously to produce an ulcer which persists until the death of the animal. Now, guinea pigs which have been infected 4 to 6 weeks beforehand and reinoculated anew, behave very differently. No nodule forms at the point of reinoculation, but from the next day or the second day this point becomes indurated and takes on a color at first violet red, and then blackish, over an area 0.5 to 1 centimeter in width. During the succeeding days the skin becomes necrotic. It soon sloughs and leaves behind it a superficial ulceration which heals rapidly and definitely, without the neighboring glands becoming swollen.

"Thus, the inoculated tubercle bacilli act quite differently when under the skin of a guinea pig *already tuberculous* than when under that of a *normal* animal. This curious effect is not peculiar to living bacilli; it is found likewise with bacilli killed either by boiling or by chemical agents."

Several of the most qualified scientists (Charrin, Baumgarten, S. Arloing, and others) hastened to repeat this experiment and did not succeed in reproducing it. In the majority of cases the infected guinea pigs into which they reinoculated bacilli died within 6 to 48 hours.

The explanation of these failures was soon furnished by Detre-Deutsch, then by della Cella,[3] Feistmantel,[4] Kraus and Grosz,[5] Römer,[6] Franz Hamburger,[7] and others.

It lies in the fact that, in animals already severely infected, a relatively small dose of bacilli, inoculated under the skin, is sufficient to produce a rapidly fatal tuberculin intoxication. Whereas, if the primary infection is not too advanced, and if it has been accomplished with just enough bacilli to produce chronic disease, not only do reinfections with suitably graduated minimal doses not cause death, but they reproduce quite exactly the clinical picture described by Robert Koch.

[3] Centralbl. f. Bakt., 1904, **36**, 12.

[4] Ibid., 1904, **36**, 282; 406.

[5] Ibid., 1908, **47**, 298.

[6] Sitzungsber. d. aerztl. Ver. z. Marburg, 1908, May 19; July 22; 1909, May 21.

[7] Beitr. z. klin. d. Tuberk., 1909, **12**, 259; 1910, **17**, 231.

It was the observation of this peculiar process,—to which the name of the *phenomenon of Koch* has been given,—that led the distinguished German bacteriologist to the discovery of *tuberculin;* but for many years, neither Robert Koch nor any other investigator grasped the importance of this phenomenon for the explanation of the mechanism of antituberculosis immunity. It required the later experiments of Calmette and Guérin,[8] of Römer,[9] of Finzi,[10] of F. Bezançon and Serbonnes,[11] and of F. Schieck,[12] to draw attention to the effects issuing from it and to show that it forms the fundamental basis upon which is to be erected the whole modern doctrine of prophylaxis against tuberculosis.

In 1903, S. Arloing[13] had noted that suspensions of tubercle bacilli, of human or bovine origin, if introduced into the circulation of animals already infected with pulmonary tuberculosis, quickly produce an edematous congestion of the lungs capable of causing death in 24 hours. The effects here are more severe than those resulting from the injection of tuberculin alone. They may manifest themselves within less than three months after the primary tuberculous infection in calves, goats and sheep, and they have been observed as early as after twenty days.

In our experiments made during 1907 and 1908, on the vaccination of cattle, I had, in collaboration with C. Guérin, drawn attention to the fact that while a normal cow always contracts an acute fatal miliary tuberculosis within 4 to 6 weeks after the intravenous inoculation of 5 mgms. of virulent bovine bacilli, cows reacting to tuberculin sustain the same inoculation without being seriously ill. After a short period of malaise and fever, everything returns apparently to normal and the tuberculous infection establishes itself in these animals in a chronic form.

"There is therefore no doubt," we wrote, "*that tuberculous animals are incomparably more resistant than normal animals to test inoculation intravenously.*"

[8] Ann. de l'Inst. Pasteur, 1907, **21**, 525; 1908, **22**, 689.

[9] Beitr. z. klin. d. Tuberk., 1909, **13**, 1; 1910, **17**, 287; 345; 357; 365; 427:—Tuberculosis, 1910, **9**, 129.

[10] Rec. de méd. vetér., 1911, **88**, 102.

[11] Bull. Soc. d'étude scient. de la tuberc., 1911/12, 2. s., 22:—Progres méd., 1912, 3. s., **28**, 293:—Ann. de méd., 1914, **1**, 129.

[12] Veröffentl. d. R. Koch Stift. z. Bekämpf. d. Tuberk., 1913, H. 5/6, 1.

[13] J. de physiol. et path. gén., 1903, **5**, 677.

"Several experiments have shown us that similar phenomena are observed in cattle artificially or spontaneously tuberculized by the digestive tract, when the animals are later inoculated with a culture of tubercle bacilli *subcutaneously*. An abscess then forms at the point of inoculation, but the neighboring glands do not swell and the abscess heals after draining.

"Clinically analogous cases are often found in man. Everyone knows that a local suppurative tuberculosis occurring in a case of pulmonary tuberculosis, ameliorates the condition of the patient and considerably increases his resistance. Inversely, it is rare that one finds cases of rapidly progressive pulmonary tuberculosis in which the individual has previously had suppurations of the glands, bones or skin, except in the case where an inopportune surgical operation has caused a blood infection.

"The idea is current at the Hôpital Saint Louis that about one-fourth of the lupus cases show characteristic auscultatory signs of pulmonary tuberculosis and that the latter generally progresses very slowly; consequently many lupus cases live to a very old age."

Applying the method which they had studied, of transcutaneous infection through the shaved or depilated skin, J. Courmont and Lesieur[14] found that, in the guinea pig, reinoculation with tubercle bacilli at least 10 to 15 days after the primary infection, produces neither local effect nor glandular involvement. Even though the reinfection be accomplished through the skin, after a subcutaneous primary infection, no new cutaneous lesion is formed.

On the other hand, A. Borrel,[15] experimenting upon rabbits with dead bacilli, found that successive subcutaneous reinoculations at intervals of 2 weeks, lead to the formation of larger and larger abscesses. Whereas a primary inoculation, of 1 mgm. for example, produces a nodule the size of a lentil, the fifth inoculation of the same dose causes the formation of an abscess as large as a hen's egg. *Reinoculations of dead bacilli produce therefore an intolerance on the part of the body just as do reinfections with living bacilli.*

In 1909 Römer published the results of his reinfection experiments upon guinea pigs and sheep. They led to the same conclusions as did ours upon cattle, namely that animals previously infected and harboring slight lesions, never contract a severe tuberculosis when

[14] Compt. rend. Soc. de biol., 1908, **64**, 882.
[15] Bull. Soc. path. exot., 1908, **1**, 420.

reinfected, even intravenously, with doses of virulent bacilli quickly fatal to controls. *The tuberculoses of reinfection always tend to the chronic form.*

Hamburger,[16] Deutsch, Veleminsky, Krauss and Hofer,[17] and later F. Schieck[18] in his experiments upon reinfection of the anterior eye chamber of the rabbit, and G. Finzi in Vallée's laboratory at Alfort, have all obtained identical results.

In 1909 likewise, F. Bezançon and Serbonnes[19] demonstrated that in the guinea pig, early reinfections, that is to say those made from the first to the fifteenth day after the primary infection, cause abscesses. Not until after the sixteenth day and particularly after the eighteenth did the true phenomenon of Koch make its appearance, characterized by necrosis. But if a considerable dose of bacilli be injected for primary infection, for example 0.5 mgm., the necrotic process is produced with earlier reinfections.

The same authors then studied, always in the guinea pig, the effects of pulmonary reinfections by inhalation. They found that lesions so produced are very different from those resulting from a primary infection. They consist, at first, in an intense capillary congestion and desquamative alveolitis. Later on, one does not see the caseation of the lesions of primary infection, but lesions of chronic alveolitis and chronic interstitial pneumonia. While the bacilli swarm in the primary alveolitis, they are very scarce in the lesions of reinfection.

On my advice, V. Grysez and Petit-Dutaillis[20] sought to determine definitely the effect of *repeated inhalations at different intervals of time,* upon the mode of evolution of pulmonary tuberculosis produced in the guinea pig by inhalation. They experimented upon 78 selected guinea pigs of medium weights (300 to 400 gms.).

The animals, confined in metal cylindrical cages, were placed in groups of 4 in a sheet iron box of about 250 cubic decimeters capacity.

[16] München. med. Wchnschr., 1908, **55**, 2702; 1909, **56**, 662.

[17] Wien. klin. Wchnschr., 1912, **25**, 574, 1111.

[18] Veröffentl. d. R. Koch Stift. z. Bekämpf. d. Tuberk., 1913, H. 5/6, 1.

[19] Ann. de méd., 1914, **1**, 129:—J. de physiol. et path. gén., 1909, **11**, 1068; 1097:—Bull. et mém. Soc. méd. d. hôp. de Par., 1910, 3. s., **29**, 232:—Bull. Soc. d'étude scient. de la tuberc., 1911/12, 2. s., 22:—Congr. français de méd., 1912, **5**, 96.

[20] Compt. rend. Soc. de biol., 1912, **73**, 728; 1913, **75**, 279.

Into the box was blown, by means of a Büchner compressed air atomizer, a very fine spray of bovine bacilli in physiological salt solution. Each inhalation treatment lasted a half hour, divided by a 10-minute intermission after the first quarter hour. With each experiment, some control animals were submitted to a single inhalation; others to 2, 3, 4, 5, 6, 7 and 8 successive inhalations repeated in the space of 2 to 36 hours; still others to inhalations repeated at longer intervals of 8, 15 or 30 days.

Of 20 control guinea pigs of the first series, subjected to a single inhalation, 19 succumbed between the seventeenth and one hundred and thirty-third day. The single one which resisted was killed after 300 days. It had only very discrete pulmonary lesions with a tracheo-bronchial adenopathy. Of the 19 others, 14 were found to have extensive caseated pulmonary lesions; 2 had miliary tuberculosis; and 3 had isolated sclerotic tubercles. Three presented true cavities, and in 15 the tuberculosis had extended to the abdominal viscera, particularly to the spleen. All, without exception, had tuberculous tracheo-bronchial glands.

On checking up the results of the experiments of the other series, they found to their surprise that in the guinea pigs given several successive inhalations at short intervals the lesions were much less extensive and less severe than in the animals which had inhaled the bacilli but once. In half of them no macroscopic lesion was to be found, either in the lungs or in the bronchial glands. Of three guinea pigs subjected to 6 inhalations within 36 hours and which were autopsied 320 and 368 days afterward, there were two which were even completely free from tuberculosis.

Contrariwise, of the 32 guinea pigs that were given inhalations at intervals of 8, 15 and 30 days, of which 11 were sacrificed after 36 to 90 days, and of which 21 died after 30 to 270 days, all were found to have large confluent caseous lesions, both pulmonary and glandular, extending in 24 to the abdominal viscera. In one animal, which died after 195 days, and which had received 3 inhalations at 8-day intervals, a lung cavity had developed.

I have gone into these experiments in some detail because they contain two very valuable and suggestive points. They teach us that *several pulmonary infections by inhalation, at short intervals, are infinitely less dangerous than a single one*, which indicates that the defensive processes establish themselves very quickly, with the

result that the body finds itself in a position to eliminate almost immediately, like foreign bodies, by its normal excretory paths, all or part of the bacilli of superinfection. But if the superinfections do not occur until some time after the entrance of the primary infecting organisms, the phenomenon of Koch manifests itself. The efforts at expulsion are more violent and remain localized at the point itself where the bacilli of superinfection are deposited. Large necrotic lesions are the result with a more widespread destruction of cells.

On the whole, what happens in the lung is exactly what occurs under the skin of animals into which small quantities of living or dead tubercle bacilli are introduced on several occasions at very short or long intervals.

Thus L. Bruyant,[21] in my laboratory, caused guinea pigs to receive during four months, at first every day, then every 3 days, a small quantity of virulent bovine tubercle bacilli, the amount being always the same, corresponding approximately to 8 bacilli. The injections were performed subcutaneously, at times into the legs, again into the abdominal wall, the site of inoculation being varied as much as possible. All of the animals died tuberculous in from 105 to 140 days later, but with lesions indicating an extraordinary resistance: serous effusions into the pleura, enormous hypertrophy of the various gland groups, a considerable cirrhotic swelling of the liver and spleen which contained large patches of sclerous induration and of greenish colored necrosis. One liver weighed 52 grams, one spleen 21 grams with not a single tubercle visible in these organs, nor in the lungs (*see Plate XII, 2 and 3*).

Therefore, superinfections repeated over a long period with very small doses of bacilli, far from aggravating and hastening the evolution of tuberculosis, give to the latter a chronic form and a very peculiar anatomo-pathological character. The follicular lesions and the tubercles give place to foci of necrosis and widespread sclerosis terminating in hypertrophic cirrhosis of the liver and in total sclerosis of the lymphatic glands and spleen.

These experimental facts clear up in a singular way those other facts of clinical observation which we were not able to explain. Bazin had noted them but Marfan[22] deserves very great credit for

[21] Compt. rend. Soc. de biol., 1911, **71**, 143.

[22] Arch. gèn. de méd., 1886, i, 423; 575.

having drawn real attention to them when in 1886 he enunciated the following *law:*

"One almost never finds pulmonary tuberculosis, at least pulmonary tuberculosis which is evident and progressive, in persons who, during childhood, have had a suppurative tuberculous adenitis of the neck and who have been completely cured of it before the age of 15 years, the cure having taken place before any other focus of tuberculosis was appreciable."

This proposition at first appeared disturbing to many clinicians. It was regarded as a sort of paradox until experimental medicine had proclaimed its accuracy and great interest.

At the present time it is no longer disputed. The more one observes and experiments, the more it asserts itself.

I had undertaken, in 1908, an inquiry among the practicing physicians of France with a view to collecting as many facts as possible for or against the *law* of Marfan.

In my letter of request I wrote, "laboratory experiments demonstrate that cattle are almost always cured when care is taken to isolate them after infecting them *a single time* by the digestive tract, whereas they are almost never cured and become rapidly tuberculous if infected *several times at short intervals*, and when left in prolonged contact with other tuberculous animals.

"The same experiments show that cattle, apparently cured, no longer react to tuberculin and often maintain perfect health, although exposed to further natural or artificial infections.

"It seems therefore that these cattle, like patients *cured* of their old tuberculous lesions, are in some manner *vaccinated.*

"It is important that we know whether in man, child and adult, immunity against tuberculosis can thus establish itself following an earlier infection, mild or severe."

Then followed a series of questions to which a large number of physicians were good enough to reply. All agreed in pointing out the rarity of severe forms of pulmonary tuberculosis in old tuberculous gland cases, in old pleuritics, and also, in a general way, in subjects who presented, particularly in childhood, a localized tuberculous lesion (bone, joint, skin, kidney, etc.)"

H. Triboulet,[23] in sharing this opinion, has expressed it very clearly. He does not hesitate to state that "localized tuberculoses at times

[23] Clinique, 1908, **3**, 340.

confer upon the body this *quid ignotum* by virtue of which, cure of the local lesion is succeeded by an evident power of resistance, on the part of the whole organism, to later tuberculous infection." P. Mével[24] in another article brings out the numerous arguments which led him to adopt the same opinion. And at the Société d'Etudes Scientifiques sur la tuberculose, Léon Bernard and Masselot[25] reported the results of a statistical study bearing upon 1046 cases of chronic pulmonary tuberculosis. In the past histories of these patients there were found only 2.2 per cent of healed suppurative cervical adenopathies; 0.09 per cent of lupus; 8.1 per cent of non-suppurative adenopathies; 2.5 per cent of various localizations. In 90.6 per cent of *adults* with pulmonary tuberculosis no old localization of tuberculosis was evident.

The *law of* Marfan is therefore valid. But its interpretation must be modified a little in the sense that the suppurative adenitis and other tuberculous localizations exercise a protective power only in so far as they still contain living bacilli in the sclerotic tissue, or bacilli temporarily attenuated in virulence. At any rate such is the case in animals artificially infected. When the focus becomes "latent" and no longer contains bacilli,—the latter having been eliminated like foreign bodies by the natural excretory routes, or destroyed *in situ* through degeneration in the midst of the calcareous deposits,—its protective action against reinfection vanishes. The body which has ceased for a greater or lesser time to react to tuberculin becomes once more liable to contract a severe tuberculosis just as though there were no previous infection. The case is exactly the same in syphilis where the only absolutely certain criterion of cure is susceptibility to contract a new chancre.

We are now therefore in a position to understand the great importance of the rôle of the *phenomenon of Koch* in the generation of anti-tuberculous immunity. For every bacillary reinfection, or more exactly for every *super-infection* (the word reinfection being capable of conveying the idea of complete cure of the primary infection) the already tuberculous organism reacts in a more intense and rapid manner to expel the invader; *it becomes more and more intolerant of the bacillus.* The latter constitutes for the cells a more and more violent poison. This intolerance and this increasing hypersensitiveness man-

[24] Bull. méd., 1908, July 22.

[25] Bull. Soc. d'étude scient. de la tuberc., 1914, June 11.

ifest themselves by a tendency to more rapid caseation, to purulent softening of the tubercles and to prompt expulsion of their contents.

This all explains to us why the forms of chronic pulmonary tuberculosis are so common in adults, in groups of people or in families where contamination is the greatest; its victims have been undergoing a whole series of more or less passive superinfections, probably since childhood. While the primary infection remained localized in a gland near to or remote from the portal of entry of the original bacilli, every later infection brought into the lymph, or into the blood, other virulent elements and, to each of them, the body reacted with a new process of elimination. On every occasion this latter took the form of a new softening of tubercles, ever more rapid and more abundant, bringing with it the destruction of tissue or organs in consequence of which the phthisical patient finally succumbs, *despite or rather by reason of the immunity which he had acquired.*

Pulmonary phthisis and chronic tuberculosis therefore afflict only those individuals previously tuberculized—most often in early life—*and rendered very resistant by a first attack, but who have not succeeded in escaping frequent or massive superinfections. On the other hand persons entirely free from tuberculosis*,—such as a large number of the rural population transplanted into the cities, or natives who inhabit the island villages of Oceania,—*die with the rapidly progressive grave forms of the disease when they happen to be exposed to abundant and repeated inoculation.*

Clinicians know, for example, how frequently it happens that *when a phthisical patient enters into a family previously free from tuberculosis, all the members of the family are quickly and severely infected.* The children die of meningitis. The adolescents or the adults succumb to miliary tuberculosis. The sole survivor, he who has brought in the disease, lives on for many years. At times even, after having sown death about him, he recovers the appearance of health, inasmuch as the superinfections undergone have increased his resistance through the fact of repeated absorptions of his own bacilli, and developed in him an intolerance to the tuberculous virus, —hence the capacity for quickly evacuating the contents of his lesions.

Among the lessons to be learned from the preceding and which should guide us with a view to a scientific and really practical orien-

tation in the campaign against tuberculosis, there is one upon the importance of which the attention of pediatricians should be centered.

We have seen that tuberculous infection, particularly during early childhood, from 1 to 5 years, seems almost inevitable,—at least in the cities. If this infection remains localized in the glandular system,—which fortunately is most often the case—and if it is not too massive, it may confer upon the child, if not an immunity, at least a state of *resistance* manifested by a propensity for eliminating the bacilli offered to it later on with new superinfections.

But what should be prevented by every means at our command is the *opportunity for him to receive frequently repeated superinfections;* since then, his capacity to eliminate bacilli becoming greater each time, increases the intolerance, causes the rapid purulent breaking down of the tuberculous foci, and makes of him inevitably a case of phthisis, more dangerous for those about him than is his disease for himself.

CHAPTER XL

FREQUENCY AND GEOGRAPHIC DISTRIBUTION OF TUBERCULOUS INFECTION.—RELATIVE SUSCEPTIBILITY OF THE VARIOUS HUMAN RACES

It has been long known that tuberculosis is very unequally distributed in the different parts of the globe and that it is particularly frequent among civilized peoples. Its diffusion is in close relationship to trade and it seems indeed that the Europeans, who are the most affected, constitute the principal vehicle of tubercle bacillus infection throughout the world.

It would greatly help our knowledge of the etiology of this disease if we might better study the manner in which it spreads and the forms which it assumes in countries previously free from the infection. This has not yet been done. From such a study we should probably be able to deduce means of prophylaxis more effective than anything we have attempted thus far. Thus, until lately, there were regarded as contagious only those individuals whose tuberculous lesions are open, particularly cases of phthisis who disseminate large numbers of bacilli about them in their sputa. Now experimentation has recently proved[1] that animals (cattle) upon which a more or less considerable resistance to tuberculous infection has been artificially conferred, or those rendered naturally resistant by a benign infection that has remained latent or occult, possess the faculty of eliminating, with their excrements, by the normal emunctories of the body (the liver and intestine), a large number of bacilli which are virulent for other animals but which, in the animals expelling them do not generate tuberculous lesions.

It was natural to suppose that this phenomenon was not peculiar to cattle and we know today in fact (Chapter XXXIII, B) that many human beings, on whom a previous benign or latent infection has conferred a relative immunity, are capable of spreading virulent bacilli about them, although themselves remaining to all appearances

[1] Ann. de l'Inst. Pasteur, 1911, **25**, 625 (Calmette and Guérin).

entirely free from infection. This being the case, one can understand that tuberculosis may be very readily propagated by European travellers,—in whom there is no objective sign of illness,—among populations previously protected through their isolation in still unexplored regions of the world.

The diagnostic procedures at our disposal today,—principally the tuberculin cuti-reaction of von Pirquet,—enable us to more precisely detect the existence of these latent or occult infections which are apparently the most dangerous sources of tuberculous contagion, because unsuspected. Thanks to these procedures we are prepared to search out, in every city, village and family, individuals contaminated with the bacilli. We can find out their ratio to the number of individuals still uninfected and thereby figure the tuberculosis index of an ethnic group, of a locality, or of a whole country. The information thus gathered is valuable not only because it serves to arouse the attention of those interested or of the public authorities and forces them to realize the necessity for defensive or protective measures, but also because it enlightens us as to the various modes of infection.

It is clear therefore why so many studies along this line have been recently undertaken. Among the most interesting in their results, I would cite those published by El. Metchnikoff, Et. Burnet and L. Tarassewitch[2] relative to the extension of tuberculosis among the Kalmucks on the steppes.

As regards countries outside of Europe, we already possess some fairly accurate statistics such as those prepared by Peiper[3] in West Africa, by Much in Jerusalem, by Römer in Argentine, by Wagon in French Guinea, by Noel Bernard, L. Koun and Ch. Meslin in Annam, by H. Gros, Ed. Sergent, Benoit, Foley, Parrot in Algeria, by Noc and Stevenel in Martinique, those which I reported following an extensive inquiry carried out in 1911 and in 1912 in the French colonies,[4] and in conclusion those of Major S. L. Cummins,[5] of G. Heim[6] and of Ziemann[7] relative to the native populations of Sudan and German Africa.

[2] Ann. de l'Inst. Pasteur, 1911, **25**, 785.

[3] Arch. f. Schiffs-u. Tropen-Hyg., 1911, **15**, 31; 1912, **16**, 431; 1914, **18**, 93; 479.

[4] Ann. de l'Inst. Pasteur, 1912, **26**, 497.

[5] Trans. Soc. Trop. Med. and Hyg., 1911/12, **5**, 245.

[6] Ztschr. f. Tuberk., 1913, **20**, 313.

[7] Centralbl. f. Bakt., 1913, **70**, 118.

The official documents published by services of public hygiene and associations against tuberculosis in the different countries inform us,—and still very imperfectly at that,—only as to relative mortality. It would be well indeed if they could be completed by surveys made in the same form. Let us hope then that such investigations will be rapidly extended and everywhere increased in number.

A. MORTALITY AND MORBIDITY FROM TUBERCULOSIS IN EUROPE

In 1906 and 1907 the comparative mortality from tuberculosis, among the principal nations making up the International Association against Tuberculosis was the following:[8]

Mortality from tuberculosis in various countries of Europe in 1908

COUNTRIES	POPULATION	TOTAL DEATHS PER 10,000 INHABITANTS	DEATHS FROM TUBERCULOSIS PER 10,000 INHABITANTS	DEATHS FROM PULMONARY TUBERCULOSIS PER 10,000 INHABITANTS	DEATHS FROM TUBERCULOSIS PER 100 DEATHS FROM ALL CAUSES
Germany*	62,849,563	180.6	17.8	15.4	10.1
England and Wales	35,348,780	147.2	15.9	11.2	10.8
Austria	27,900,924	225.0	30.4		13.5
Belgium	7,386,444	165.1	13.0	10.1	8.3
Denmark (towns)	2,635,000	154.4	17.6	13.3	11.7
Scotland	4,826,587	161.3	19.6	12.6	12.2
Spain	19,712,585	250.2	18.5	13.6	7.4
France	39,196,328	190.0	22.6	18.7	12.8
Greece (cities of more than 10,000 inhabitants)	2,631,952	238.3	33.9	24.8	14.4
Hungary	20,786,278	244.4	37.0		15.1
Ireland	4,371,455	175.9	25.8	19.5	14.8
Italy	34,129,304	225.6	16.6	10.5	7.4
Norway	2,321,575	142.9	24.4	18.8	19.3
Holland	5,786,232	150.2	16.2	12.0	11.7
Portugal	5,423,132	226.4	11.8	9.9	7.8
Roumania (32 cities)	6,771,722	258.7	30.9	25.1	11.9
Sweden (cities)	5,377,713	141.1	26.7	20.7	19.0
Switzerland	3,554,672	162.3	24.1	17.3	15.5

* Exclusive of Mecklenburg-Schwerin and Mecklenburg-Strelitz.

[8] From statistics published by the Internat. Bureau of Public Hygiene, and by Hamel of the K. K. Gesundhtsamte at Berlin.

The principal European nations were classed in 1908 in the following order from the point of view of their mortality from tuberculosis per 10,000 inhabitants:

Portugal	11.8
Belgium	13.0
England	15.9
Holland	16.2
Italy	16.6
Germany	17.8
Spain	18.5
Scotland	19.6
France	22.6
Switzerland	24.1
Norway	24.4
Ireland	25.8
Austria	30.4
Hungary	37.0

And, in the same year 1908, the proportion of deaths from *pulmonary tuberculosis* per 100 deaths from all forms of tuberculosis was the following for the different countries of which we possess official statistics:

Deaths from pulmonary tuberculosis among 100 deaths from all forms of tuberculosis

Italy	63.4
Scotland	64.2
England and Wales	70.4
Switzerland	71.8
Spain	73.3
Holland	73.9
Ireland	75.4
Norway	77.2
Belgium	77.7
France	82.9
Portugal	83.8
Germany	86.3
Hungary	87.3

The cities, particularly the very large ones, have everywhere a higher mortality then do the rural districts.

While for the whole of Germany for example, the figure was 1.78 per 1000 inhabitants in 1908, it rose in Berlin to 2.18 and in the district of Allenstein (Eastern Prussia) was only 0.97.

By the mortality statistics of Prussia,[9] 60,871 persons died from

[9] Med. stat. Nachrichten, 1911–12, fasc. 2 König. Statistischen Landesamtes, Berlin.

tuberculosis in 1909 and 60,479 in 1910, or respectively 9.11 and 9.48 per cent of deaths from all causes and 15.59 to 15.29 per 10,000 inhabitants.

In the cities the mortality from tuberculosis according to age was in 1910:

Up to 1 year	1,032
From 1 to 15 years	4,449
From 15 to 30 years	10,152
From 30 to 60 years	14,481
From 60 to 70 years	2,110
Above 70 years	759
Total	32,983

and in the rural districts:

Up to 1 year	1,235
From 1 to 15 years	3,504
From 15 to 30 years	8,027
From 30 to 60 years	11,392
From 60 to 70 years	2,487
Above 70 years	850
Total	27,495

According to Behla,[10] in Prussia, the tuberculosis mortality per 10,000 inhabitants fell from 30.95 in 1876 to 14.58 in 1912. But the decrease occurs exclusively among individuals of more than 15 years. Below this age the figures remain stationary, especially from 5 to 10 years.

At the time of the last International Conference on Tuberculosis which met in Berlin in October 1913, Hamel called attention to how variable is the ratio, by age and country, between the average number of pulmonary tuberculoses and of tuberculous localizations in organs other than the lung.

In Prussia for example, between 1 and 15 years, pulmonary tuberculosis causes 53 per cent of the total of deaths from tuberculosis, and other localizations 47 per cent.

From 15 to 60 years the figures are 93 per cent for pulmonary tuberculosis and but 7 per cent for tuberculosis of other organs.

In England, forms other than pulmonary are much more frequent, especially in children where there are 34 pulmonary tuberculoses

[10] Berl. klin. Wchnschr., 1913, **50**, 1950.

against 66 in other organs. The situation is practically the same in Sweden, Norway and in Denmark.

In England, including Wales, the statistics of the Local Govern-

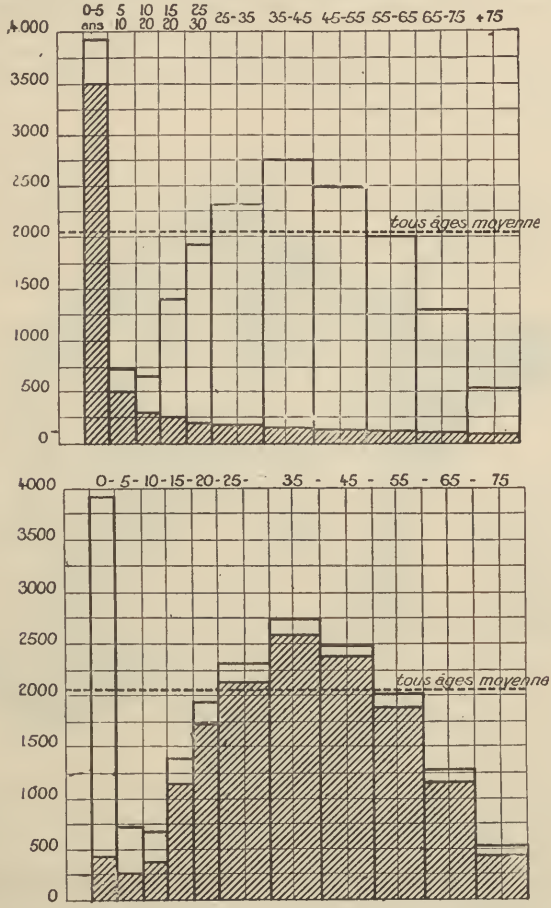

FIG. 29. ANNUAL TUBERCULOSIS MORTALITY, PER MILLION INHABITANTS, OF ALL AGES, DURING THE DECADE 1891–1900, IN ENGLAND AND WALES (FROM SHERIDAN DELÉPINE)

ment Board show for 1909 a proportion of 7.4 per cent dying from tuberculosis per 100 deaths. For the city of London alone, this proportion amounts to 9.3 per cent. In Scotland it is 8.1 and in Ireland 10.8 per cent. This gives a general average of 8.8 per cent of deaths

from all causes in the whole of the United Kingdom, the population of which is 44,590,000 inhabitants.

Figure (No. 29), taken from Sh. Delépine, gives the annual death rate from 1891 to 1900 from pulmonary phthisis and from all the various forms of tuberculosis per million inhabitants, and for the different ages. Very much the same curve might be drawn for all the European countries.

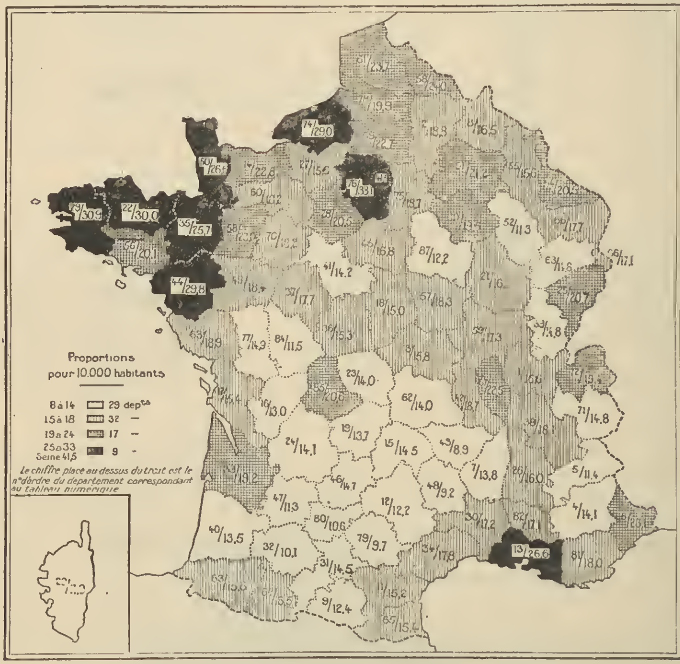

FIG. 30. MORTALITY FROM TUBERCULOSIS IN FRANCE IN 1911

In France, the total number of deaths from tuberculosis of the lungs or meninges reported in the statistics of the Ministry of the Interior, for the year 1909, was 84,918 in the population of 39,196,328, and 19.27 per 100 deaths from all causes, that is 2.16 per 1000 inhabitants. In 1913 the same statistics give 84,443 deaths from tuberculosis (17.76 per cent of deaths from all causes, or 2.13 per 1000 inhabitants).

But these figures should in reality be considerably higher, since without doubt a good part of the 115,781 other deaths due to pulmonary affections should be charged to tuberculous infection. The

deaths from pulmonary affections not classified under the heading tuberculosis were divided as follows for the year 1909:

Acute bronchitis	16,615
Chronic bronchitis	18,389
Pneumonia	38,708
Other affections of the respiratory passages	42,069
Total	115,781

At Paris, with a population of 2,722,731, there were in 1909, 11,685 deaths from tuberculosis and, since 1880, this figure has scarcely varied. The proportion to other causes of death tends rather to increase. It is established at about 25 per cent.

Percentage of deaths from tuberculosis at Paris

YEAR	DEATHS FROM TUBERCULOSIS	TOTAL DEATHS	PROPORTION PER 100 DEATHS FROM ALL CAUSES
1880	11,023	55,706	19.78
1890	12,586	54,566	23.06
1900	12,548	51,725	24.25
1905	11,952	47,843	25.00
1910	11,723	45,814	25.58
1913	11,119	45,355	24.51

While the general mortality falls steadily, that from tuberculosis tends to remain stationary or even to increase!

Unfortunately one may assert that the same holds true for the whole of France and the following tabulation offers proof:

Mortality from pulmonary tuberculosis alone, per 10,000 inhabitants

	1891–95	1906	1910	1911
The whole of France		18.2	17.9	18.0
Paris	40.9	37.4	35.3	34.2
Cities of 100 to 500,000 inhabitants	28.1	28.8	26.0	26.6
Cities of 30 to 100,000 inhabitants	23.2	27.6	27.5	27.0
Cities of 20 to 30,000 inhabitants	20.7	24.5	24.7	25.0
Cities of 10 to 20,000 inhabitants	19.2	22.7	23.2	23.4
Cities of 5 to 10,000 inhabitants	16.7	18.6	18.7	18.7
All cities of more than 30,000	30.8	31.3	29.5	29.2
All cities of less than 30,000	18.4	21.3	21.7	21.8
All cities	25.5	27.1	26.3	26.2
Communities of less than 5000 inhabitants		13.1	12.8	12.9

It is seen therefore that the large cities are twice as much infected as the country at large, but the situation of the latter is none the less disturbing, since the development of general hygienic measures is progressively lowering the total death rate from the aggregate of disease in a very definite way, while the tuberculosis mortality is not appreciably affected.[11]

The above figures afford sufficient evidence as to the influence of dense social grouping upon the creation of conditions favorable for the propagation of tuberculous disease.

But this influence appears even more obvious when one refers to statistics of tuberculin tests such as those which I was able to collect with V. Grysez and R. Letulle at Lille, from June 1, 1911 to December 31, 1913, in a total of 2108 persons of all ages, *exclusive of hospital groups.* They indicate that above the age of 15 years, 88 per cent give a positive cuti-reaction, that is to say, *among 100 individuals above 15 years of age and of healthy appearance, about 88 harbor the tubercle bacillus to a varying extent!*

At Vienna, von Pirquet found analogous figures, even a little higher. The same is true for Budapest. At Prague, Ganghofner[12] tested with the cuti-reaction 552 children in his hospital. Among 462 non-tuberculous, he found 179, or 28 per cent who reacted; among 90 clinically tuberculous, 82 or 91 per cent gave a positive reaction.

At the Emperor Francis Joseph Hospital, at Vienna, 400 children aged from 3 months to 14 years, from the poorest families of the city, gave 186 positive reactions or 46.5 per cent (Ottokar Gruener).[13]

In Norway, tuberculous infection is about as frequent[14] and early as in France or Germany. B. Overland carried out a very conclusive investigation on this subject in the schools of Bergen. Of 843 children from 7 to 15 years old, 28.07 per cent gave a positive cuti-reaction to tuberculin. Those aged 10 years reacted in the proportion of 51 per cent. Of the infected, 71.46 per cent came from families where one or more members had tuberculosis.

In their study of the epidemiology of tuberculosis among the Kalmucks, a pastoral folk of the neighborhood of the Volga, El.

[11] Presse méd., 1913, i, 497 (Discussion of this subject by E. Fuster).

[12] Wien. klin. Wchnschr., 1908, **21**, 1403.

[13] Ibid., 1908, **21**, 986.

[14] Ztschr. f. Tuberk., 1913, **20**, 252.

Metchnikoff, Et. Burnet and Tarassewitch find that in the central part of the steppes, the region where the inhabitants have but little intercourse with the cities, the proportion of adults reacting positively to tuberculin is 69.4 per cent for men and only 30.06 per cent for adult women, whereas in the outer parts of the territory, where commercial relations with the Russian population are very active, 95.7 per cent of adult men and 88.5 per cent of women give a positive reaction.

It must therefore be concluded that, *in the urban centers of Europe and among the rural populations which have frequent relations with them, at least nine-tenths of the individuals reaching adult age have not succeeded in escaping tuberculous contamination.*

B. ASIA

Next to Europe, mortality from tuberculosis is highest in Asia. This is not surprising, inasmuch as this part of the world is inhabited, in its fertile regions, by a very dense population whose ancient civilization has brought about a congestion often greater than among ourselves.

We have no statistics as to tuberculosis in China. Morache, who resided for a long time at Pekin, observed phthisis in all stages and called attention to its extreme frequency. It is, he says, the chief cause of death among the poorer classes. At Shanghai it causes 60 per cent of deaths in the Chinese hospital. H. Dold[15] studied the mortality statistics of this city from 1900 to 1915 and reports that the Chinese die from tuberculous infection considerably more than do the foreigners. The average mortality for them is 2.7 per 1000 (general mortality 18.2 per 1000), while for foreigners it is 2.2 per 1000 (general mortality 17.4 per 1000). This difference is accounted for indeed by the poor hygienic conditions in which the native population lives; one cannot deduce from it that the Chinese are more susceptible to tuberculosis than are Europeans.

Phthisis is very common also at Canton, Amoy and at Hong Kong in the native quarters.

The same is true of Japan, Siam, Singapore, Java, Sumatra, and in the Philippine Islands (11.7 per cent of deaths in Manila) according to Isaac W. Brewer, Musgrave and Sison;[16] in British India

[15] Deutsch. med. Wchnschr., 1915, **41**, 1038.
[16] Philippine J. Sci., 1910, **5**, 313.

(13.51 of deaths at the general hospital at Madras from 1907 to 1911), and also in Ceylon. It is stated by Tholozan that it is rare in Persia, although Becker[17] asserts that since 1905 it has greatly increased following the arrival of fairly large number of Europeans. Lancereaux thinks that this is due, not to the climate, but to the manner of living of the Persians, who for 6 months of the year sleep in the open air upon roofs or in gardens and, during the cold season, live in well ventilated dwellings.

In Syria, Anatolia and Armenia, the severe forms of pulmonary tuberculosis are infrequent. They are said to be observed only exceptionally in the interior of the country; the Fellahs for example give scarcely any positive reactions to tuberculin. But in the cities near the coast, at Smyrna, Beirut, Jerusalem, at Jaffa and among the Jews of the Yemen district, according to Hans Much[18] phthisis is very common and the children are often affected with characteristic adenitis. At Jerusalem the maximum percentage of positive reactions (23 per cent) is observed in subjects from 11 to 14 years of age.

We are more exactly informed as regards our French Asiatic colonies.

In French India, P. Gouzien[19] finds from 1890 to 1900, in a total of 43,317 deaths, 868, or an average of 17.2 per 1000, caused by tuberculosis.

In Indo-China, the natives know this disease very well, and call it *binh ho lao* (*consumptive disease with cough*). It was already very widespread before the conquest. The physicians who make vaccination tours have reported it as extremely common even in the villages of the interior of the country (Hénaff).

At the native hospital of Hanoi, Monzels tested 293 individuals with the cuti-reaction. He found 146 positive reactions, or 49.8 per cent. At the civil prison in the same city, among 436 subjects of more than 15 years, Gauducheau found 147, or 37 per cent, who reacted.

In Annam, Noel Bernard, L. Koun and Ch. Meslin undertook similar studies among various groups of people (in the schools, native troops, native officials, tradesmen, laborers, and peasants, prisoners

[17] Tuberculosis, 1915, **14,** 149.
[18] Ibid., 1913, **12,** 415.
[19] Ann. de méd. navale et coloniale, 1904, **7,** 543.

and prostitutes). Infection among adults was found more frequently in the higher social classes than among the lower: 70.8 per cent of positive tuberculin reactions among the former, 60.3 per cent among the latter. This difference is probably due to the fact that the laborers and peasants live an active life out of doors, while the more educated classes, the officials, lead a sedentary existence, without any physical exercise, at times in badly ventilated places. The prisoners, it is true, work out of doors during the day, but they are confined during the night in too small quarters and sleep side by side on the same camp bed. They give positive reactions up to 83.1 per cent.

In Cochin China, cuti-reactions performed by Brau on 57 individuals from 15 to 20 years of age, gave 24.6 per cent of infected cases, and at Cambodia, among 411 subjects, Crossouard finds only 19 positive reactions or 4.6 per cent. Furthermore it is commonly said that tuberculosis is very rare in this country. It has existed only since the Chinese and Annamite invasion. But very few Europeans are there.

C. AFRICA

The native people of Africa have been spared from tuberculosis in so far as they have escaped subjection. The virus has been introduced and continues to spread among them, borne by Arabs and Europeans whether conquerors or traders. Cairo, Alexandria, Tripoli, Tunis, Bone, Algiers, Constantine, have today a tuberculosis mortality approximately equal to that of Barcelona, Marseilles or Naples. The same is true of the islands of the Atlantic (Cape Verde Islands, Canary Islands, Azores). At Madeira even, which was proposed as place of cure, tuberculous infection is very common among the native population (G. Railfiet).[20]

North Africa, however, back from the coast, is still but little contaminated.

The same may be said for the south of Africa, as well as for the east and west coasts. The further one goes back from the sea and from localities frequented by Europeans, the rarer becomes tubercle bacillus infection. It is scarcely encountered as far in as the Upper Nile among the negroes of Darfur, and has appeared in the native

[20] Rev. de la tuberc., 1911, **8**, 86.

river villages of the Niger, of Chad, of the Congo, of the Zambezi only following the immigration of foreign elements. Major S. L. Cummins and also A. Balfour[21] state that the natives of the Sudan and those of the Bahr-el-Gazal appear to contract tuberculosis only when in contact with the posts frequented by Europeans.

In the Belgian Congo, in a total of 79 autopsies (sleeping sickness excluded) performed during 18 months at the hospital for negroes of Leopoldville, R. Mouchet[22] found lesions of tuberculosis 29 times, or in 36.7 per cent.

At Cameroons, H. Ziemann[23] performed tuberculin cuti-reactions upon a considerable number of natives and immigrants belonging to various races, and found that the infection is relatively rare among the Dualas. The percentage of positive reactions was from 3 to 4.6 per cent among the young aborigines and pupils of the missions of less than 16 years; 6.6 per cent among the prisoner Dualas; 3.8 per cent among Bantu soldiers; and only 0.9 per cent among Bantu negroes. On the contrary, it was found much higher among the Hottentots who had been transplanted from Southwest Africa to Cameroons (14 per cent among men, 22 per cent among women, 15 per cent among children); higher still among the Haoussas, (26.3 and 18 per cent) and among the immigrant Syrians. The Haoussas are the Mohammedan traders who overrun Africa in all directions and propagate disease, among others, syphilis. They certainly are a contributing factor in the diffusion of tuberculosis. The hygiene of the colonies of Africa should therefore take into consideration these people who are already much more tuberculous than the non-roving blacks.

Tuberculin cuti-reactions indicate that in Senegal, among individuals of more than fifteen years, 15.2 per cent are infected; in Guinea 5 per cent; in German East Africa, O. Peiper states that among 98 blacks at Kilwa, 17 per cent are infected with the bacillus and among 79 immigrated Indians he finds 25.4 per cent; on the Ivory Coast the proportion is 8.4 per cent; at Madagascar 7.1 per cent; in the Seychelles 17.1 per cent. But in the old French colony of Reunion, the number of infected cases amounts to 81 per cent, a figure very close to that of European cities.

[21] Rep. Wellcome Trop. Research Inst., 1911, **4**, (A), 286.

[22] Bull. Soc. path. exot., 1913, **6**, 55.

[23] Centralbl. f. Bakt., 1913, **70**, 118.

In Algeria, Edm. Sergent and Benoit find a percentage of 40 among Arabs of 30 to 70 years who occupy the spurs of the Atlas mountains and who as farm workers live in constant contact with colonies of Europeans. Foley obtained 22 per cent of positive reactions among Arabs and Berbers living in the oasis of Figuig and in the oases of the Sahara in the extreme south of Oran. The general average of infected cases among the native adults, in Algeria, is about 52.8 per cent.

In Egypt, tuberculosis is very common among the native born and still more so among the negroes come from Sudan or from Darfur. At the Hospital of Kasr-El-Ain, at Cairo, 80 per cent of the total deaths from tuberculosis are among negroes, although Europeans are far from spared. In the city of Alexandria alone the tuberculosis mortality rate is 23 per 10,000 inhabitants. The disease occurs throughout the country the same as in Europe as to severity and forms. The Egyptian climate has no favorable influence upon its progress or cure. Instead, it is rather bad for tuberculous cases during 8 to 9 months of the year (Valassopoulo).

D. OCEANIA

In Australia, tuberculosis, although less frequent than in Europe, is nevertheless very widespread particularly in the cities. At Melbourne for example, of 3468 deaths, 328 are due to phthisis (Bird). It has been spreading since the middle of the last century with great intensity among the aborigines of the interior, as well as in Tasmania and in New Zealand where it causes more than half of the deaths.

On the contrary, the disease is still rare in the Malay and Polynesian Islands whose native populations, up to the present time, have had but little contact with Europeans or Asiatics. But it propagates itself with extreme rapidity wherever commercial dealings and immigration become active. Whereas in New Guinea, for example, 10 per cent of native deaths were already due to tuberculosis in 1909–1910, and while at the German station of Yap, in the Western Carolines, of 785 native born patients treated in the hospital, 14 had pulmonary tuberculosis, and 41 various tuberculoses localized in other organs, at Ponape (Eastern Carolines) there was only one case of pulmonary tuberculosis among 1742 patients (Kersten, Salecker).[24]

[24] Arch. f. Schiffs-u. Tropen-Hyg., 1915, 9, 101; 369

W. E. Musgrave and G. A. Sison[25] state that tuberculosis has become very common in the Philippine Islands. At Manila, in 1908, among 100,000 natives, there were 486 deaths (J. W. Brewer) and among 600 autopsies, Gilman and Andrews found 32 to 40 per cent of subjects who bore active lesions.

In New Caledonia, tuberculosis has been propagated chiefly by the convicts. "Scarcely 10 years after the establishment of the prison," wrote Mesnard in 1903,[26] "tuberculosis had spread enough to especially attract the attention of the natives by the deaths which it was causing among them. Without their taking account of the extension of the disease, one symptom particularly impressed them: the emaciation, the consumption. It appears certain in fact that, among the native islanders, tuberculosis has a special tendency to progress rapidly but quietly and to end in pulmonary phthisis. It is rare that one sees other tuberculous manifestations. It may be stated that nowadays the native succumbs, in general, either to tuberculosis or to leprosy."

The same may be said of the New Hebrides and of all the Polynesian Islands recently colonized. At Tahiti, in the Loyalty Islands, in the Marquesas, in the Carolines, at Samoa, tuberculosis is or is rapidly becoming the principal agent of depopulation. It is spreading among the natives with terrifying rapidity. The acute forms running their course in 3 to 4 months are the most commonly observed. In children only the glandular forms are found, bone or joint tuberculosis rarely.

It would seem that the severity of the infection, on each island, is proportional to the number of Europeans. It is established too that the Canaques transported to the cities of the western shores of America, there die very rapidly of tuberculosis. A few years ago an English speculator took two thousand natives from the Marquesas to Lima (Peru). In less than 18 months three-quarters of them were dead of phthisis.

E. AMERICAN CONTINENT

South America, from the southernmost regions of Patagonia to the Isthmus of Panama, has concealed foci of tuberculosis which decimate the native population. These foci were created by Euro-

[25] Philippine J. Sci., 1910, **5**, 313.

[26] Ann. d'hyg. et de méd. colon., 1903, **6**, 597.

pean colonists. The large cities of Buenos Aires and Montevideo have a tuberculosis mortality higher than that of Berlin. Davidson[27] is authority for the statement that, between the ages of 20 and 40, twice as many Argentinian native-born as foreign immigrants die of phthisis. The central provinces of the Argentine, and the Pacific coasts of Peru and Chili (Westenhoffer)[28] are equally invaded. Phthisis is very common at Guayaquil, at Valparaiso and even at Lima, and also at La Paz (Bolivia), in spite of the altitude of this locality (3717 meters). It is also very common in Brazil and is there found more frequently and in more grave form among the Brazilians than among the foreigners (Clemente Ferreira).[29]

In the Central American States and the Antilles it is likewise very widespread.

We possess some accurate information as to the extent of infection in the French Antilles, thanks to many tuberculin cuti-reactions performed there during 1912 by Noc, Stévenel, and Sauzeau de Puyberneau.

At Guadeloupe, among 257 subjects of more than 15 years, 113, or 40 per cent, gave a positive reaction. At Basse-Terre, the proportion of infected individuals in the insane asylum is 45 per cent. At Désirade, of 301 subjects, 109 reacted, or 36.2 per cent, and at Martinique, 101 of 177 subjects, or 57 per cent. The general average for the whole of the Antilles is about 41 per cent.

According to Noc, tuberculosis develops much more rapidly among the creoles than among the European colonists. In the past the negro slaves were rarely affected. It was to the interest of the planter to keep them well housed, nourished and clothed and they were given plenty of medical care in order to avoid diseases which might reduce their working capacity. Freedom has in a certain measure changed the conditions of their existence to their detriment and has given them phthisis. They now contaminate one another in the dirty huts in which they live in cities or villages, and their badly kept dwellings form quarters which it is very difficult to keep in a hygienic state. In addition, alcoholism makes terrible ravages among them and contributes notably to diminishing their resistance.

In Mexico, tuberculous infection annually accounts for between

[27] Lancet, 1910, ii, 233.

[28] Berl. klin. Wchnschr., 1911, **48**, 1063; 1105; 1158; 1207; 1259; 2036.

[29] Tuberculosis, 1915, **14**, 15.

6 and 8 per cent of deaths from all causes. At Mexico City, it was 9.31 for the period between 1891 and 1898. This is a relatively low figure (Ed. Liceaga).

In the United States, the official statistics give 11.15 in 1907 and 11.32 in 1908 per 100 deaths, and respectively 1.83 and 1.73 per 1000 inhabitants. These figures are higher than those of Germany and a little lower than those of France.

Tuberculosis, writes Thomas D. Coleman,[30] was unknown to the Indians of North America before European colonization. Today it constitutes the most important factor in the destruction of their race and causes 66 per cent of deaths! The negroes of North America likewise show a much higher mortality than the white race and this death rate has increased in particularly large degree since the suppression of slavery.

At New Orleans for example, from 1897 to 1907, the average death rate among the negroes was 56.7, while that of the whites was 23.4 per 10,000. The proportion of deaths from tuberculosis to the total number of deaths was for each race, 17.2 per 100 for the blacks, while only 12 per 100 for the whites.

F. THE RELATIVE SUSCEPTIBILITY OF THE VARIOUS HUMAN RACES TO TUBERCULOUS INFECTION

It is seen from the foregoing that no human race escapes tuberculosis and that the disease is particularly widespread among the most recently civilized peoples, whereas the native-born populations of countries where civilization has not yet penetrated are practically free from the disease. This does not mean that they possess any degree of natural immunity, indeed it is quite the contrary, since the disease strikes them with terrible severity once they are brought into contact with infection from foreign peoples who are bearers of bacilli.

The influence of climate upon the greater or lesser frequency of infection shows itself, in spite of anything that one may say or write on this subject, to be absolutely nil. Tuberculosis is as widespread and serious among the Esquimaux or Laplanders as among the negroes of the Congo or the Canaques of the Hebrides. If the latter are destroyed in relatively smaller number than are the

[30] *Tuberculosis*, edit. by Arnold Klebs, New York, 1909.

Europeans, it is due exclusively to their custom of living in fairly small groups or to the nomad life of certain among them, as a result of which massive infections and superinfections are avoided.

In Greenland, for example, according to Meldorf, all men of 25 years are tuberculous or present the stigmata of tuberculosis and the disease generally assumes a very benign form. The ill survive for decades.

A very suggestive investigation of this subject was carried out in 1910 in the Swedish provinces inhabited by the Laplanders. In the province of Norr-Botten, the population at the time was 3530, of whom 2292 were nomads and 1238 residents. Of the total there were examined 1770 subjects, or half the Laplander population; 1,066 were nomads and 704 residents. The results compiled by F. Block revealed the existence of pulmonary lesions clinically detectable in 2.25 per cent of the nomads and in 3.55 per cent of the residents, and individuals of more than 50 years were the most frequently affected.

In the commune of Kiruna, although a mountainous and almost uninhabitated region, tuberculosis seems to have made its appearance in 1900 with the exploitation of an iron mine. There were then 312 inhabitants, and 10 years later there were more than 8000. Kiruna is situated at 67.51° north latitude, 1412 kilometers from Stockholm, and north of the Arctic Circle. G. Neander examined there 2000 individuals in 1910. The majority were miners or of their families. Of 998 adults of both sexes, there were 56 definitely tuberculous (5.6 per cent) and 25 suspects (2.5 per cent). Among 1002 children 8 were definitely tuberculous, but 335 (33.5 per cent) had swollen lymphatic glands or suppurative adenitis. This finding suggested the idea of dividing the children into two categories: those born at Kiruna and whose age did not exceed 9 or 10 years, and those from elsewhere. Of 566 born at Kiruna, 204 had adenitis, while among the 436 immigrant children there were 131 or 36 per cent as against 30 per cent. It appears that the infection was rather more quickly spread among the folk originally resident than in the immigrating families who had brought in the disease.

It has often been remarked that in cities where tuberculosis is very widespread the Jewish race gives a mortality rate perceptibly lower than that of the population as a whole. Germain Sée,[31]

[31] Bull. Acad. méd., 1891, **55**, 238.

A.-L.-J. Béraud,[32] Fishberg,[33] and other authors have called attention to this fact, and the United States statistics are particularly interesting in this regard. According to an official inquiry made by Fishberg, bearing upon 10,618 Jewish families and comprising 60,030 individuals, the mortality from tuberculosis was 36.57 in 1000 deaths among Jews and 34.02 among Jewesses. On the other hand, for the whole population of Massachusetts in 1888, corresponding figures for all people other than Jews were 129.22 for men and 146.97 for women. Another investigation made by Fishberg at New York in 1901, in a part of the city peopled with Irish, Italians and Greeks, and where the Jews made up only a negligible minority, shows that the mortality from tuberculosis varies between 40 and 50 per 10,000 non-Jewish inhabitants, while varying from only 11 to 21 for the Jews.

At Vienna,[34] from 1901 to 1903, the mortality from pulmonary tuberculosis was 38.8 per 10,000 among the Catholic inhabitants, 24.6 for the Protestants and only 13.1 for Jews.

At Lemberg, where the Jews live under very wretched conditions, their death rate from tuberculosis is 30.64 per 10,000, as against 63.51 for the Christians. Furthermore the figure for Cracow is 20.49 for the Jews, against 66.41 for the Christians, and at Budapest in 1905, respectively 21.93 against 46.01.

At London,[35] the Jews live for the most part in the Whitechapel district where they follow all sorts of generally unhealthy occupations. Their mortality from 1891 to 1900 was but 12.3 per 10,000 inhabitants, against 17.9 for the population as a whole.

Tostivint and Remlinger[36] at Tunis, from January 1, 1895, to December 31, 1899, found 1017 deaths from tuberculosis among 13,151 deaths among the Mohammedan population (7.73 per cent). During the same period, the percentage of deaths from tuberculosis was 3.96 for Europeans (French, Italians, Greeks, etc.) and only 1.23 (34 in 2744) among the Jews.

All of these statistics seem then to indicate that the Jewish race is distinctly less subject than other races to tuberculous infection. But this is so only in appearance, since if one studies in each country

[32] Thèse, Bordeaux, 1897.
[33] Med. Record, 1908, **74,** 1077.
[34] Veröffentl. d. Bureau fur Statistik der Juden, Berl., 1908, H4.
[35] Brit. Med. J., 1908, i, 1001.
[36] Compt. rend. Soc. de biol., 1900, **52,** 833

not only the causes of mortality but also the causes of morbidity, it is seen that tuberculosis is as common among the Jews as among the Christians.

Thus, Maurice Fishberg[37] studied the children of 217 New York Jewish families, aided by charitable organizations and in which the father or mother was tuberculous. He found that among 692 children, 65 were manifestly infected. Of the 65 children, 13 had bone or joint lesions, 4 had Pott's disease, 2 spina ventosa, 19 pulmonary tuberculosis and 25 tracheo-bronchial adenopathy.

In these same families, 188 children had died under 14 years; 30 of them of tuberculous meningitis.

The reports of the Henry Phipps Institute of Philadelphia also indicate that the number of Jews who come there to receive care, or who react to tuberculin, is proportionally approximately the same as that of individuals of other white races (Cheinisse).[38] The same fact is to be observed in Europe when one refers to the statistics of public institutions.

If the tuberculosis death rate for the Jews in the cities is less than that of the Christians, while the morbidity is practically the same, the cause lies in the fact that the Jews, whose entire life is passed almost exclusively in urban groups and very seldom in the country, are, by the nature of their life, exposed from the earliest age to mild infection, the vaccinating power of which is today well known. And if the rapidly fatal forms are more rare among them, it is due on the one hand to the resistance which they acquire by virtue of their early and benign infection, and on the other to the fact that alcoholism and physical overstrain exert upon them only exceptionally the depressant action which is so important a factor in the aggravation of the disease among others of the white race, and still more among negroes.

Tuberculosis attacks all human races. When considerable differences in tuberculosis mortality exist between populations of different countries, or in the same country among populations of different origins (for example, Negroes, Indians, Japanese or Chinese, Northern or Southern Europeans, half breeds, etc.), they result simply from the fact that tubercle bacillus infection has been implanted in them over a longer or shorter period of time and that the circumstances of con-

[37] Ann. Rep. Henry Phipps Inst., Philadelphia, 1906/12.
[38] Semaine méd., 1908, **28**, 613.

tagion vary, infection being at times more rare, or again more massive or frequent, according to the peculiar conditions of life. Peoples who have been longest protected through isolation upon islands, by difficulties of commercial intercourse or by the relative absence of crowding in their groups, are found to be the most susceptible. Such is the case with the Kalmucks and the young men of Bosnia and Herzegovinia drafted into Austrian regiments. Among them the disease most often assumes severe and rapidly progressive forms when they are exposed to frequent contagion. This happens almost always in the case of native-born Africans transplanted into the large centers of Europe or America.

People contaminated for centuries, collected into large social groups, more exposed to infection from early age through living in more or less close and prolonged contact with diseased individuals who spread bacilli,—such as the Jews,—are on the contrary more resistant. The disease ordinarily assumes in them the chronic slowly developing forms. But almost all individuals are infected and those who, during childhood or adolescence, have by chance escaped a benign or severe infection, have a susceptibility to the virus which equals that of individuals of races free from infection.

The extreme diffusion of tuberculosis throughout the world and the facility with which it propagates itself not only through individuals affected with open lesions, but also through the immense number of apparently healthy individuals who are at one and the same time *carriers* and (through their various excretions) distributors *of bacilli*, lead us therefore to regard the total eradication of tuberculous infection as impossible, perhaps even undesirable.

On the contrary, our hope should be to render this infection harmless through early vaccination and through the increase of measures of prophylaxis designed *to prevent massive or frequent inoculation*, a danger particularly great for all human beings of every race.

CHAPTER XLI

PASSIVE IMMUNITY

Attempts at Antituberculosis Serotherapy

We have seen in a previous chapter (XXXVII), in speaking of the formation of the *antibodies* whose presence in the serum of tuberculous subjects can be detected by the so-called fixation reaction of Bordet-Gengou, that it is possible to stimulate a more abundant production of these antibodies by repeated injections of certain antigens (tuberculin, dead or living bacilli), and that therein lies the essential goal of *tuberculin therapy.*

It is generally granted that a sort of parallelism exists between the antibody content of the serum of an animal or tuberculous human being and the activity of his means of defense against infection by the tubercle bacillus. This hypothesis does not seem to be strictly correct, since lesions frequently continue their evolution even when antibodies are present in large amount. The fact that the antibodies disappear toward the end of the disease and that as a rule their quantity increases in proportion to the defensive effort on the part of the body, indicates that they reveal themselves rather as an evidence (*témoins*) of cellular reactions against infection. But that in itself is enough to justify the search for them and to incite us to discover suitable means for increasing their production.

This is the chief aim of present day *antituberculosis serotherapy.*

Until late years, the existence and probable rôle of the antibodies having escaped the attention of experimenters, the endeavor was to prepare sera to restrict the development of the tuberculosis and to neutralize *in vitro* and *in vivo* the toxic products derived from the bacillus. They tried to adapt to the treatment of tuberculous infection the principle of the method so successfully instituted by von Behring and Em. Roux to prevent and cure diphtheria with the serum of vaccinated animals. Innumerable attempts were made with this idea in view and all ended in complete failure.

They deserve to be recalled however, if only to enable future workers to dash through the stages where their predecessors were forced to pause and thus to reach more quickly the ground which they propose to explore.

A. ATTEMPTS AT ANTITUBERCULOSIS SEROTHERAPY.—MODE OF PREPARATION OF SERA

The first attempts at specific serotherapy against tuberculous infection were made by Ch. Richet and Héricourt[1] in 1888. In a series of notes at the Société de Biologie these scientists showed that the evolution of tuberculosis due to a human type bacillus in various animals like the dog, monkey and also man, may be favorably influenced by the injection of serum from a normal dog, or from a dog previously inoculated, first with avian bacilli and then with human bacilli. But the authors themselves said that the action was only temporary ,and their efforts were never successful in accomplishing a cure.

Richet and Héricourt had likewise tried to hypervaccinate an ass with intravenous injections of human bacilli. But its serum showed no immunizing power, either preventive or curative. The same was true of other animals prepared by means of tuberculin, but with the difference that the serum of the latter was obviously poisonous; it hastened the progress of the tuberculosis and induced febrile reactions similar to tuberculin reactions.

A few years later, Babès and Proca[2] injected dogs successively with avian and human tuberculin, and afterward with virulent cultures of avian and human strains of bacilli. The serum of these animals gives much the same results as those obtained with the serum of normal dogs. Nevertheless in two cases there seemed to be a certain favorable effect upon dogs and rabbits.

Other similar attempts, unsuccessful or doubtful in outcome, were later made by Vicquerat[3] with the serum of a mule injected with cultures from glycerin broth; by Von Schweinitz and Dorset with sera of the mule, ass, or horse, prepared by injections of an aqueous extract of bacilli; by Redon and Chenot[4] with the serum of

[1] Compt. rend. Soc. de biol., 1889, **41,** 157; 1890, **42,** 316; 325; 627; 630; 1895, **47,** 15:—Compt. rend. Acad. des sci., 1892, **115,** 842.

[2] Ztschr. f. Hyg., 1896, **23,** 331.

[3] Centralbl. f. Bakt., 1899, **26,** 293.

[4] Compt. rend. Soc. de biol., 1895, **47,** 493.

goats inoculated with extracts of tuberculous organs; and by Maxutow, likewise with the serum of goats treated with an alcoholic and glycerinated extract of tuberculous nodules from a case of perlsucht.

Later came further discouraging attempts on the part of Boinet with the serum of goats treated with raw tuberculin; of Niemann who also inoculated goats, first with raw tuberculin, then with precipitated tuberculin and finally with living tubercle bacilli. The serum of these animals seemed to have some effect upon the guinea pig.

Frisch tried to immunize horses with the tuberculin T. R. of Koch. Löwenstein inoculated goats intravenously with a suspension of avirulent human bacilli, then with other bacilli which were more virulent, and succeeded thus in obtaining a serum whose agglutinating titre was 1 in 5000. But it did not in any way modify the virulence of the bacilli, even after 24 hours of contact at 37°, no matter whether supplemented or not with the fresh serum of a normal or tuberculous animal. This serum possessed no preventive or curative property against experimental tuberculosis.

P. Baumgarten and C. Hegler[5] succeeded in vaccinating a bullock with human bacilli and later inoculated him, five times in succession, with bovine bacilli without there being any appreciable symptoms of illness. The tuberculin test was always negative. The serum of this bullock was injected as a preventive into a calf and enabled the latter to resist an inoculation of virus which severely infected a second control calf, and a third calf in which the serum was not employed until after the virulent inoculation. This third animal received in all 70 cc. of serum in several injections during the two weeks which followed its infection. From a curative standpoint therefore the serum was not capable of arresting the progress of tuberculosis.

Lannelongue, Achard and Gaillard[6] tried the serum of the ass and of the horse which had been injected with extraction products of tubercle bacilli killed by heating and macerated in weakly acidulated water. The extract was afterward treated with pure acetic acid and the precipitate washed, then redissolved in a weakly alkaline solution.

[5] Berl. klin. Wehnschr., 1905, **42**, 55.

[6] Compt. rend. Acad. des sci., 1906, **142**, 1479; 1908, **147**, 612.

The first trials with this serum were upon guinea pigs. Those which had been treated showed a somewhat lower death rate and the lesions were apparently more circumscribed than in the controls. In tuberculous patients, Kuss did not find any specific action upon the lesions, but the serum was not harmful.

Ferran, Daremberg, Prioleau and Paquin, also turned to the ass for antitoxic serum; Trudeau and Baldwin to the ass, sheep, rabbit and hen. Paterson and then Auclair tried the serum of hens inoculated intravenously with human bacilli.

S. Arloing and L. Guinard,[7] with a view chiefly to the preparation of an antitoxic serum, inoculated 6 goats separately with the following substances: (1) Virulent bacilli; (2) Raw tuberculin from the Pasteur Institute; (3) Raw tuberculin prepared by themselves; (4) Tuberculin obtained by the decoction at 85° to 90° of bacilli taken from cultures; (5) Tuberculin prepared with the broth of a culture rid of its alcohol-precipitable portion; and (6) Tuberculin obtained from the alcohol-precipitable portion.

The sera of these goats were all found almost equally inactive. Still it was thought that the three first had weak *antituberculin* properties.

Similar attempts made by Maffucci and di Vestea were never successful.

It seems evident then that *all sera of animals prepared either with tuberculin, or with bacilli, are devoid of any efficacy, as well for tuberculous patients as for experimental tuberculosis.*

Nevertheless Néporoshny[8] thinks that he has had fairly satisfactory results in the guinea pig, from the therapeutic and even from the preventive standpoint, with the serum of a dog treated in the following rather complicated manner:

To begin with the animal is immunized with tuberculous endotoxin. To this end there is used a highly agglutinating horse serum, which is made to act upon tubercle bacilli. As soon as the dog tolerates well a large quantity of this endotoxin inoculated subcutaneously, injections of fat-free bacilli are made into the veins and into the peritoneum. Later there are injected bacilli which are not rid of fat, but which are killed with chloroform. Finally, to complete the immunization, which requires at least 8 months, the dog receives living

[7] Internat. Congr. on Med., 13th, Paris, 1900.

[8] Arch. d. sci. biol. de Petrograd, 1908, **12**, No. 4.

and virulent bacilli intravenously or into the peritoneum. Afterwards it is bled.

With this serum the author states that he has been able to successfully treat guinea pigs, the proportion of cures being 54.5 per cent when the treatment, begun 5 to 6 weeks after inoculation of the virus, could be continued for five and a half months. In the guinea pigs which had undergone treatment from the first week, the proportion of animals cured was said to be 96 per cent!

The anatomo-pathological lesions observed in the treated guinea pigs were such as are produced by killed cultures, or those become avirulent with age. It seems that, under the influence of the serum of Néporoshny, the body reacts to virulent tubercle bacilli as if it were dealing with dead bacilli.

Von Behring,[9] with all his persevering search for methods of active and passive immunization against tuberculosis, did not succeed in obtaining a practically utilizable antituberculous serum.

It seemed therefore after so many unavailing efforts that all hope of success would have to be abandoned. But the complexity of the problem could not dishearten the workers, all the more since physicians, feeling themselves so often disarmed before their patients were freely offering themselves for experimentation with the new forms of medication which were proposed.

Among the so-called *antituberculous* sera thus introduced into therapy before having undergone a sufficiently prolonged and rigorous testing in the laboratory, as would have been desirable, the best known and the most commonly utilized are,—or have been,—those of Maragliano, of Marmorek, of Vallée, and that of Ruppel and Rickmann, the latter put on the market by the firm of Hoechst; and finally those of Bruschettini and of A. Jousset.

It is not within the scope of this book to study the practical applications of the different sera and tuberculins to the treatment of human tuberculosis. These sera are of interest here therefore only from the strictly biological point of view.

1. *Serum of Maragliano*

In 1895 Maragliano[10] began his observations as to the effects of a certain serum upon tuberculous patients. This serum he prepared

[9] Deutsch. med. Wchnschr., 1904, **30**, 193.

[10] Berl. klin. Wchnschr., 1899, **36**, 1073:—Compt. rend. Soc. de biol., 1897, **49**, 309:—Ann. d. Ist. Maragliano p. la cura d. tuberc., 1906/07, **2**, 133.

by injecting subcutaneously and intravenously into various animals (horse, bullock, goat) a mixture of broths from young cultures and of aqueous extracts (precipitated with alcohol) of virulent bacilli (*tossina precipitata*).

A little later, he modified his method of preparation by injecting these same animals, in addition, with the product obtained by grinding bacilli killed with heat.

The serum thus obtained is called *bacteriolysin* and is said to have at one and the same time antitoxic, bactericidal, bacteriolytic and agglutinating properties. The antitoxic power should be measured by calculating the quantity of bacillary extract toxic for 100 gms. of normal guinea pig,—this quantity is said to be about 1 cc. A serum of which 1 cc. protects 100 gms. of normal guinea pig against a fatal dose of bacillary extract is said to contain 100 antitoxic units. But it must be recognized that the bacillary extracts vary in toxicity and are ordinarily much weaker than Maragliano states. It frequently happens that normal guinea pigs will tolerate 5 to 10 cc. with impunity. The result is that the very principle upon which the antitoxic activity of the serum is measured is extremely variable.

The bactericidal and bacteriolytic action are demonstrated, according to Maragliano, on the one hand, by the fact that the bacilli do not develop either upon specific gelatin serum nor upon broth supplemented with 30 per cent of this serum and, on the other hand, because admixture *in vitro* produces a "bacteriolysis" of the bacterial cells.

In this regard, J. Teissier (of Lyons) states that if a mixture of bacilli and the serum be injected into the anterior eye-chamber of a rabbit, there are no harmful effects.

However, as I said before, no one has succeeded in obtaining positive results on repeating this experiment.

While there is no doubt as to the fact that the serum of Maragliano, like the majority of those prepared by other authors, is definitely agglutinating, it cannot be denied that, as a rule at least, it contains no antibody. If its effects upon patients and also upon tuberculous animals are in a certain measure favorable, it must be regarded as acting like an *antigen*, since it contains tuberculin and more or less diluted bacillary products. It would favor then the formation *in vivo* of antibodies, but would in no way realize the *passive immunity* sought in the use of antituberculous sera.

Its therapeutic value has been attested by some clinicians, especially in Italy, (Marzagalli, Geordano, Bartieri, Figari, Cambiaso, and others), and in France by J. Teissier (of Lyons). It has been stoutly denied by L. Flick and Landis after protracted experiments carried out according to the directions of Maragliano himself at the Henry Phipps Institute at Philadelphia. It was also disputed by L. Guinard after a series of conscientious attempts at the sanatorium of Bligny, and by Maffucci and di Vestea[11] who tested it from the experimental side.

The "bacteriolysin" of Maragliano is employed in patients, at the Genoa clinic, by subcutaneous injection, with an initial dose of 1 cubic centimeter repeated every two days. After 10 such treatments followed by 10 days of rest, 2, 3, 4, and 5 cubic centimeters are injected, each dose being repeated 10 times. This treatment moreover is recommended by its originator only for cases of incipient tuberculosis, or where there are progressive lesions not yet beyond the second stage of Turban.

If, as often happens the injections produce general and local reactions which are very similar to tuberculin reactions, care must be taken to cut down the dose and prolong the interval in order to avoid complications (congestions, hemoptyses, etc.).

2. Serum of Marmorek

In a communication to the Academy of Medicine of Paris in 1903, A. Marmorek[12] reported the encouraging results which he had obtained, principally in the treatment of surgical tuberculoses, with a serum prepared by vaccinating horses with filtrates of young cultures, in which the so-called primary (*primitifs*) bacilli grow rapidly in the form of a thin film, are not yet provided with their complete waxy fatty ectoplasm, and do not therefore for the most part hold the Ziehl stain. As culture media he utilizes a "leucotoxic" calf serum mixed with glycerin broth. In order to render this serum "leucotoxic" he injects the animal beforehand with a suitable quantity of peritoneal exudate rich in mononuclear leucocytes and with an emulsion of guinea pig liver.

Tubercle bacilli, grown upon such media, produce a toxin of

[11] Centralbl. f. Bakt., 1896, **19**, 208.

[12] Bull. Acad. méd., 1903, **50**, 332; 465; 480:—Med. klin., 1906, **2**, 58:—Berl. klin. Wchnschr., 1907, **44**, 18.

moderate activity, of which 8 to 10 cc. will kill a 400 gm. guinea pig in about one week But these animals can be easily immunized against this toxin by injecting them 6 or 8 times at suitable intervals with a dose of 5 cc. They are said to be then capable of tolerating with impunity the inoculation of one or two drops of a "slightly opalescent" suspension of virulent culture. They should in any event survive notably longer than the controls.

Marmorek thinks that his serum neutralizes the true toxin of the tubercle bacillus, which we are unable to produce artificially outside the living organism and which, according to him, is not tuberculin. A single fact appears certain: it is that this serum, which indeed contains only very little antibody, is ordinarily harmless, or at least is incapable of causing anything worse than the accidents of anaphylaxis. To avoid them, Marmorek recommends that it be employed first in small daily doses subcutaneously (2 to 10 cc.), later by the rectum (5 to 20 cc.) in enemas repeated 2 or 3 times per week. But the effects are then uncertain or nil, inasmuch as the work of Hamburger and other investigators indicate that *antitoxins do not pass through a healthy rectal mucosa.*

Yet a fairly large number of physicians and surgeons, particularly in Germany, Austria, Switzerland, Italy and in France, consider that they have obtained good results, principally in certain forms of surgical tuberculoses such as fistulae of the perineum (Turban, Frey, Hoffa, Jacobsohn, Lewin, G. Sohenker, Wohlberg, Dubard, Schmoller, Monod,[13] and others).

Some of them attribute these favorable effects to the fact that this serum contains a very small amount of tuberculin. Its use then is equivalent to injecting minimal doses of the latter substance subcutaneously or into the rectum.

I found with L. Massol that Marmorek's serum does not in any way neutralize the effects of tuberculin, and certain specimens which we studied did not contain any antibody. Grüner,[14] in Escherich's laboratory, made the same observation and found no difference between the action of this serum and that of normal horse serum upon tuberculin *in vitro* and *in vivo.*

Be that as it may, the numerous articles published as to Marmorek's serum during ten years attest that it can be used therapeu-

[13] Bull. Acad. méd., 1907, **57**, 122.
[14] Wien. klin. Wchnschr., 1908, **21**, 986.

tically without danger of serious accidents and that, under certain circumstances, its use has been followed by surprisingly rapid improvement. It seems however that pulmonary tuberculoses as a rule derive no benefit from it.

3. Serum of Vallée

Beginning in 1909, Vallée[15] of Alfort had L. Guinard at the Sanatorium Bligny, Rénon, Castaigne, F. X. Gouraud, Boureille and a few other French clinicians test a serum which he obtained by vaccinating horses with intravenous injections of avirulent tubercle bacilli of equine origin, and afterward with successive injections, properly spaced and in increasing amounts (up to 250 milligrams at a time), of human bacilli. The horses thus prepared received afterward, likewise intravenously, decanted cultures (non-filtered) of human bacilli, and bacillary endotoxins extracted from unheated bacterial bodies by protracted grinding. The grinding was done in darkness, in an atmosphere of hydrogen in order to avoid oxidation processes.

The serum, collected one month after the last injection, heated at 56°C. for one hour on three consecutive days, and aged in an ice-box for several months in order to reduce the chances of anaphylaxis to the minimum, then has, says Vallée, antitoxic, anti-endotoxic and weakly agglutinating properties (1 to 50 at the maximum). It should favorably influence the evolution of the disease in tuberculous cattle and should contain specific antibodies in abundance.

Tests carried out upon human patients are said to have given encouraging results in about one-fifth of the cases. The results however were frequently nil and at times even unfavorable. It appears, according to Léon Bernard and J. Paraf,[16] and R. Debré and Porak,[17] that the events observed following injections are not attributable to the special activity of the serum of Vallée but to inherent and little known qualities of the body fluids of the tuberculous. The latter show in fact a quite special propensity for reacting violently to serum inoculations and these reactions manifest themselves intensely also with normal sera or with therapeutic sera of any sort whatever.

[15] Bull. Soc. centr. de méd. vétér., 1906, **60**, 407:—Ann. de l'Inst. Pasteur, 1909, **23**, 585; 665.

[16] Bull. Soc. d'étude scient. de la tuberc., 1911, May.

[17] Presse méd., 1912, ii, 809.

Through the kindness of Vallée, I have had the opportunity of studying experimentally with L. Massol two samples of his serum. We found that it contained antibodies in appreciable amount: one cubic centimeter deviated 1.6 cc. of normal guinea pig complement, of which the minimal hemolytic dose was 0.0075 cc. This would mean that 1 cc. deviates 213 minimal hemolytic doses of complement or is equivalent to 213 *units of antibody.*

On the other hand, the samples which we studied did not possess any antitoxic power against tuberculin. Four tuberculous guinea pigs artificially infected 6 weeks beforehand, received subcutaneously mixtures of 0.5 cc. of raw tuberculin and 19.5 cc. of Vallée's serum: all were dead in less than 6 hours. Other tuberculous guinea pigs which received 0.1 cc. of raw tuberculin and 10 cc. of serum intraperitoneally, also succumbed and after the same interval as the controls inoculated with the same quantity of tuberculin mixed with normal serum.

4. Serum of Ruppel and Rickmann

Ruppel and Rickmann[18] prepared a serum which is put upon the market by the firm of Hoechst. They started on the perfectly logical principle that normal animals, as used by the majority of investigators, are incapable of producing tuberculous anti-endotoxins, inasmuch as they are not poisoned by these endotoxins, whereas on the contrary, tuberculous animals, very sensitive to these endotoxins, can react by producing antagonistic substances. They begin therefore by injecting living and virulent human bacilli intravenously into cattle or mules. They wait until tuberculous lesions are produced, then load their animals with increasing quantities of tuberculin, bacillary extracts and living bacilli until they no longer react to tuberculin. Thus they succeed in obtaining "immune sera" whose properties have been well studied experimentally and which show themselves rich in specific agglutinins and particularly rich in antibodies, but incapable of neutralizing the toxic action of tuberculin for tuberculous guinea pigs. But they are said to neutralize (in the same tuberculous guinea pigs) tuberculins free of albumoses and those of bacillary extracts.

According to Ruppel and Rickmann, a serum of which 1 cc.

[18] Ztschr. f. Immunitätsforsch., 1910, 6, 344.

gives the fixation reaction of Bordet-Gengou in the presence of 0.01 cc. of "standardtuberculine," contains one unit of antibody. The unit of antigen is the quantity of antigen contained in 0.01 cc. of "standardtuberculine." One gram of dried human bacilli or 1 gram of dried extract of O T or of T R (of Robert Koch) contains 62,500 units of antigen.

Through the courtesy of the originators I was able to study in 1914, with L. Massol, some samples of their serum. By titrating the antibodies we found that 0.001 cc. of one serum deviated 0.075 cc. of normal guinea pig complement in the presence of the antigen B^2 which we were using (peptonated bacillary extract). One cubic centimeter therefore deviates 15,000 minimal hemolytic doses of complement, whereas 1 cc. of Vallée's serum, of which we have already spoken, deviates only 213 doses. (In my experience, neither the serum of Ruppel and Rickmann, nor that of Vallée, has any curative action upon tuberculosis in the guinea pig.)

As yet we have no definite information as to the results of some tests of this serum, carried out principally by Sobotta, in the treatment of human tuberculosis. However it has been studied by F. Meyer[19] from the standpoint of sensitizing tubercle bacilli after the general technique of Besredka. According to Meyer, guinea pigs tuberculized with 1 mgm. of human bacilli (weighed dry), and then treated 10 to 17 days after the infection with sensitized bacilli, survived considerably longer (more than 5½ months) than the controls, which died in 5 to 6 weeks.

5. *Serum of Bruschettini*

Bruschettini[20] of Genoa prepares a serum which he uses in mixture with a special bacterial vaccine for the treatment of patients. The animals which yield this serum are immunized with increasing quantities of endotoxins obtained by producing a pleural exudate in rabbits through injections of aleuronat and bacilli, later by injecting them intravenously with bacilli heated to 60° for two hours.

This serum-vaccine has been tried by the author in Italy and at the Brompton Hospital in London, upon tuberculous patients, with very uncertain results.

[19] Berl. klin. Wchnschr., 1910, **47**, 926.

[20] Internat. Congr. on Tuberculosis, 10th, Rome, 1912:—Tuberculosis, 1914, **13**, 432.

6. *The serum of Jousset*

A. Jousset[21] immunizes horses by injecting them intravenously or subcutaneously with progressively increasing doses of a human bacillus of attenuated virulence for the guinea pig. The details of his technique have not yet been published.

The efficacy of the serum obtained cannot, according to the originator, be measured by experiments *in vitro*. He states that no parallelism exists between the content of coagulins, opsonins and antibodies and its therapeutic potency against the bacillus. For this reason he has more confidence in direct experimentation upon patients. Jousset believes that he has seen unmistakable improvement in cases which he has treated, particularly during the periods of bacillemia early in the disease.

7. *Immunizing bodies IK of C. Spengler*

Although we are not dealing here with a serum, properly speaking, the immunizing bodies of C. Spengler should be mentioned in connection with attempts at antituberculosis serotherapy, since they too aim at a passive immunity.

The *Immunkörper*, or IK, of Carl Spengler[22] are prepared by a special technique from the whole blood of immunized rabbits. The author starts on a theory, completely opposed to all those accepted up to the present, that red blood cells play an essential rôle in the phenomena of immunity, and that in artificially immunized animals the immunizing substances are accumulated chiefly in the stroma of these red cells. The immunizing substances must therefore be set free by dissolving the whole blood and they are obtained by immediately putting a certain quantity of blood collected in a syringe from the marginal ear vein of a vaccinated rabbit into a 0.3 per cent lactic acid solution.

To vaccinate the rabbits, C. Spengler begins by inoculating a small quantity of human type tubercle bacilli *directly into the muscle.* The latter, according to him, of all the tissues in the body, is one of the least favorable to multiplication of the bacilli, even the most

[21] Compt. rend. de la Caisse Nationale des recherches scient., 1912–1913; Bull. Acad. méd., 1918, **79**, 432:—J. méd. français, 1918, **7**, 158.

[22] Deutsch. med. Wchnschr., 1908, **34**, 1620:—*Tuberkulose und Syphilis Arbeiten,* Davos, 1913, Erfurt.

virulent. The animal so prepared is capable of receiving with impunity, after a few weeks, one or several successive doses of virulent bacilli under the skin of the axillary pocket. The doses may be of the type *humano-longus*, virulent for the rabbit, or of the bovine type.

The blood of rabbits thus vaccinated against tuberculosis may be mixed with that of other rabbits vaccinated against the various bacteria of secondary infection. One may even prepare rabbits with the patient's own bacteria (tubercle bacilli and organisms of secondary infection isolated from the patient himself), and thus obtain what Spengler calls the *complete antituberculous* IK.

The stock solution (*solution mère*) of blood should always be diluted to 1 in *one hundred thousand*, that is to say 1 cc. of this solution represents 0.00001 cc. of the original blood, or of the mixture of original bloods. It is put on the market in this dilution. It is a colorless liquid, weakly acid, which does not cloud either on dilution or with Esbach's reagent, and which contains traces of methemoglobin.

For therapeutic use, it is diluted with an antiseptic acid solution (*sodium chloride 5 gms.; carbolic acid, 5 gms.; lactic acid, 3 gms.; distilled water*, 1000 cc.). Dilutions are thus made up to 1 in one million, or 1 in ten million, etc. One need only mix 9 cc. of the antiseptic diluent with 1 cc. of the stock solution or of the preceding solution. The successive dilutions are numbered from I to VII, No. I being a 1 to 10 dilution of the stock solution, so that 1 cc. represents the *millionth part* of 1 cc. of the original immune blood.

C. Spengler attributes to these IK a *lytic* power against the tubercle bacillus and an *antitoxic* power against tuberculin.

The *lytic power* is measured by injecting a 1 in one million dilution of stock solution of IK into a normal rabbit, and inoculating 24 hours later, under the skin of the ear, a small quantity of virulent culture. If the bacilli are absorbed, the solution of IK possesses the desired lytic action. The author takes no account of the fact that the bacilli have not really been *dissolved*, but only quite simply absorbed and borne here and there in the body by the lymph and the leucocytes.

As a control of the *antitoxic* power C. Spengler takes 1 cubic centimeter of a 1 in one million dilution of the original 1 in one hundred thousand dilution for example, and adds to it the minimal lethal dose of tuberculin for a tuberculous guinea pig of 250 gms.

weight. The mixture is injected subcutaneously. If the animal survives, the 1 cc. of the 1 in one million dilution of IK contains 1 antitoxic unit. One cubic centimeter of the 1 in one hundred thousand dilution (original stock solution) of the same IK should therefore contain 1 million units.

The treatment of patients with the IK at *Davos* is begun with dilution VI and carried along by the following plan, with an injection every ten days:

1st injection	1.0 cc. of No. VI
2nd injection	1.0 cc. of No. V
3rd injection	1.0 cc. of No. IV
4th injection	1.0 cc. of No. III
5th injection	1.0 cc. of No. II
6th injection	1.0 cc. of No. I
7th injection	0.2 cc. of original solution
8th injection	0.5 cc. of original solution
9th injection	0.8 cc. of original solution
10th injection	1.0 cc. of original solution

The injections are generally very well borne, even by febrile patients, and are said to have therapeutic effects which ordinarily are favorable.

The latter statement is, however, stoutly disputed by many clinicians. Roepke[23] finds the effects to be only those to be obtained with pure physiological salt solution. They were nil in 65 patients at his sanatorium at *Stadtwald-Melsungen*. H. Weicker and B. Bandelier[24] at *Görbersdorf*, Wallerstein at *Moscow*, Kerle[25] at the sanatorium of *Müllrose*, and many other clinicians have not been more fortunate. Sophie Fuchs-Wolfring,[26] and Castaigne and Bénazet,[27] on the contrary, report a certain number of facts suggesting that the treatment with IK results favorably. But upon analysis one feels that, although it may be claimed that the immunizing bodies are not harmful, there is nothing to demonstrate that the increases in weight and improvements in physical signs on the part

[23] Deutsch. med. Wchnschr., 1909, **35**, 1831.
[24] Ibid., 1909, **35**, 1833.
[25] Berl. klin Wchnschr., 1910, **47**, 627.
[26] Rev. de la tuberc., 1912, 2, s., **9**, 1.
[27] J. méd. français, 1914, **7**, 295: 301.

of certain patients should be attributed to the therapy. In order to be convinced of this, it would be necessary to study experimentally the effects of the substance upon animals. This has been neglected up to the present time. Landemann[28] however has performed some laboratory experiments with the IK found upon the market, against his bacillary extract called *Tuberculol.* He found that they were *devoid of all neutralizing action in vivo and in vitro.*

B. PROPERTIES OF ANTITUBERCULOSIS SERA

The antituberculosis sera prepared by different workers possess in varying degree the property of agglutinating tubercle bacilli and of precipitating tuberculins. They also contain, as a rule, antibodies in variable amount. As I have already stated, the tendency at the present time is to attribute any favorable effects particularly to the latter, although experimentally,—at least in tuberculosis of the guinea pig,—these favorable effects are not evident.

a. Agglutinating power

Tubercle bacilli, depending upon their origin, are more or less agglutinable by the sera of tuberculous human beings or animals. It is rare that the agglutination titer of the latter exceeds 1 in 20 (S. Arloing and P. Courmont). On the other hand, in animals which have received repeated intravenous injections of bacillary extracts, dead bacilli or living bacilli, the agglutinating strength increases and may reach a considerable figure. With C. Guérin,[29] I found that cattle, hypervaccinated to the extent of having received 2.1 grams of bovine bacilli during 2 years, furnished sera capable of agglutinating cultures of our bile treated bacillus at 1 in 15,000, and cultures of ordinary bovine bacilli at 1 in 2000.

This serum, so rich in agglutinins, was proved experimentally to be lacking in all preventive or therapeutic properties. It does not seem therefore that the agglutinins play any rôle whatever in the defense of the body against tuberculous infection. At most they are to be regarded only as an "evidence" of this defense.

[28] Berl. klin. Wchnschr., 1908, **45**, 2017.

[29] Compt. rend. Acad. des sci., 1910, **151**, 32.

b. Power to precipitate tuberculins

In 1909, I demonstrated with L. Massol[30] that the sera of our hypervaccinated cattle precipitated solutions of various tuberculins. Since then this property has been regularly observed, not only with antituberculous sera, but also with sera and various body fluids from patients. The intensity of the precipitation reaction varies greatly. At times it is very strong, so much so that 0.01 cc. of serum suffices to produce an obvious clouding, after 1 or 2 hours in the incubator, with 1 cc. of a solution of an aqueous bacillary extract. With sera from hypervaccinated animals it may be almost entirely lacking. It is evident therefore that *there is no correlation between precipitation and the degree of immunity.*

Furthermore, the reaction *is not specific.* It reveals apparently the liberation of a more or less large amount of globulins by the simple act of diluting the sera, since an identical precipitation is often observed when sera of subjects suffering from various infectious diseases (typhoid fever, pneumonia, exanthematous typhus) are diluted in about 5 volumes of distilled water (*see Chapter XXX, F*).

The precipitate thus obtained with the tuberculous sera is not made up of tuberculin, since after several successive washings and centrifugations, it proves inactive in tuberculous subjects, whether on subcutaneous injection, by the cuti- or ophthalmo-reaction, or even by intracerebral inoculation into tuberculous guinea pigs.

Nor is it constituted of sensitized tuberculin, inasmuch as doses of precipitate corresponding to the initial tuberculin, do not absorb complement and do not give the reaction of Bordet-Gengou.

On the contrary, the same serum, if treated with a quantity of tuberculin capable of producing the maximum precipitate, or with lesser amounts, and from which the precipitate has been separated after centrifugation, contains approximately all the tuberculin originally present. With dilutions of this serum freed of precipitate, the same tuberculin reactions (*subcutaneous, cutaneous or ophthalmic, intracerebral toxicity*) are obtained as with dilutions of tuberculin of the same titer. Therefore *it does not contain antituberculin.*

[30] Compt. rend. Acad. des sci., 1909, **149**, 760.

c. *Antibody content*

In the present state of our knowledge the search for *antibodies* in antituberculous sera appears infinitely more interesting from the point of view of determining the respective values of these sera. This is particularly true because a large number of recent publications tend to show that a fairly close parallelism exists between the formation of these antibodies in the blood of tuberculous subjects and the strength of the processes of defense on the part of the body against tubercle bacillus infection.

Experiments which I published with L. Massol[31] on this subject brought out the fact that it is relatively easy to obtain sera rich in tuberculous antibodies by injecting repeated spaced doses of bacillary extract into either normal horses or cattle. However, the production of these antibodies depends upon how the injection is made: thus a normal horse receives two successive doses of 20 cc. of bacillary extract (containing 2 per cent dry extract), at 12 days interval; the antibodies suddenly appear in abundance in the serum of the blood withdrawn on the twelfth day following the second injection. If the injections of bacillary extract are continued further in larger doses (40 to 100 cc.) with the same intervals, the antibodies disappear entirely and no more are produced.

Contrariwise, if another horse is injected simply with repeated small doses of bacillary extract (2 cc. diluted with 1 cc. of physiological salt solution) daily for 20 days, the animal, from the second day after the last injection, furnishes a serum containing more antibodies than that of the horse treated by the first method.

It is essential to carefully define the conditions for obtaining the complement deviation reaction with the various antigens (tuberculins, bacillary extracts, bacilli, etc.,) and the tuberculous antibodies. Now we have found that the quantity of complement fixed is roughly proportional to the quantities of antigen and antibodies brought together.

The antibody in a given reaction should never be in excess, in relation to the antigen, since fixation may not occur.

In studying sera of animals during the course of vaccination, we have noticed that certain among them acquire the curious property

[31] Compt. rend. Soc. de biol., 1909, **67**, 528; 1910, **68**, 48; 224; 1911, **71**, 191; 341; 1912, **72**, 15; 658; 1912, **73**, 120.

of preventing the fixation reaction from taking place, in other words of *inhibiting* it, when a small amount of the serum is added to a mixture of antigen + antibody, before the addition of complement. The inhibitory property does not manifest itself if the inhibiting serum is introduced into the mixture of antigen + antibody after the complement is added (*Chapter XXXVII*).

If, in a fixation reaction performed in the presence of an inhibiting serum, the amounts of antigen (bacillary extract) or of antibody are varied, employing uniformly a dose of 0.05 cc. of inhibiting serum, it is seen that excess of antibody has no effect. On the contrary, in proportion as the antigen is increased, the fixation reaction manifests itself anew and inhibition is obscured.

We have moreover demonstrated experimentally that the inhibiting sera themselves contain antibodies whose presence is masked by the inhibiting property. The inhibitory substance (*inhibitrice*) therefore masks the existence of antibodies up to the point where its affinity for antigen is satisfied, and this affinity for antigen is greater than that of the antibodies. There is here a phenomenon comparable to that observed with diphtheria antitoxins, for example, whose affinity is not the same for the *toxin* as for the *toxones* and *toxoids* of Ehrlich.

On the strength of these findings, we determined to divide antituberculous sera into two groups:

1. Those sera which contain antibodies solely. They give the reaction of Bordet-Gengou in the presence of the smallest doses of antigen. When added in large excess with the same dose of antigen they do not modify the fixation.

2. Those sera which contain both antibodies and inhibitory substance (*inhibitrice*). They give the reaction of Bordet-Gengou only in the presence of large amounts of antigen. Employed in excess they do not deviate complement.

Antibody-containing sera, when brought into contact with bacilli, do not all behave in the same manner; some (those of the first group) attach their antibodies indifferently to the soluble antigen of the medium or to that adherent to the bacilli; others (those of the second group) fix their antibodies exclusively upon the living or dead bacilli, for example upon suspensions of bacilli remaining from the preparation of aqueous bacillary extracts, or upon the antigen extracted by macerating dry bacilli in a 10 per cent solution of Witte's peptone on a water bath for 48 hours at 65°C.

The technique of fixation reactions has been already described (*see Chapter XXXVII*). I shall only recall here that the attention of workers should be constantly drawn on the one hand to the necessity of always using a dose of hemolytic serum 10 to 20 times larger than the minimal dose necessary to produce hemolysis and, on the other, to the desirability of employing only such complement as has been titrated immediately before the tests.

As *antigen* the raw tuberculin of Koch may be used; but it contains inactive substances which impair the fixation reaction.

Tuberculin purified by precipitation with alcohol cannot be used. The same is true of broths filtered and evaporated after removal of the bacilli.

Peptonated bacillary extract B^2, or dry bacilli killed by boiling at 100°C. and suspended in physiological salt solution in a proportion of 1 to 1000, make the best antigens. In all cases they permit the fixation of antibodies contained in sera, even when the latter contain inhibitory substances.

When the different elements necessary for the fixation reaction have been properly prepared and titrated, the search for antibodies and their quantitative determination can be patterned after that for antigens. *The unit of antibody contained in a serum may be represented by the quantity of antibody capable of deviating a minimal dose of complement.*

The so-called *antituberculous* sera of which we have spoken above do not all contain antibodies. Some samples among those we have studied failed to show a trace. Others, like that of Ruppel and Rickmann, contained up to 15,000 units per cubic centimeter on titration; that of our hypervaccinated cattle gave 2500 units; that of Vallée only 213 units, etc.

Now, when the therapeutic action of these various sera upon laboratory animals and cattle is studied experimentally, it is found now and then that the *sera richest in antibodies hasten the evolution of the disease instead of retarding it.* Perhaps these unfavorable effects are due to the fact, which I discovered with C. Guérin, that the sera set loose the bacilli in the body, inasmuch as they cause in part their expulsion by the hepatico-intestinal path.

C. MODE OF ACTION AND THERAPEUTIC VALUE OF THE ANTI-TUBERCULOSIS SERA

In the recent medical literature there is found a large number of observations by clinicians who seem favorably disposed to the use of one or another antituberculous serum. But it can not be scientifically established that the improvement or apparent cure of the favorable cases is really due to specific properties of these sera. It is a well known fact that in phthisiotherapy, at any moment, the most surprising changes for the better may intervene following upon all sorts of circumstances and modifications. Simple rest, the fresh air cure, change of regime, at times within a few days, will modify or arrest the evolution of a tuberculosis which promised to be serious.

Every one knows too how frequently a tuberculous lesion is apparently cured under the influence of small doses of tuberculin repeated frequently enough and over sufficiently long periods. And indeed there seems to be no doubt that *certain so-called "antituberculous" sera act as though they contained very small doses of tuberculin.* So it is with the serum of Marmorek which ordinarily contains no antibodies, and with that of Maragliano. In this way then they may be of use. But their beneficial action is often counteracted by the accidents of anaphylaxis provoked by repeated injections, and also by the well known and quite special sensitiveness on the part of the tuberculous to foreign sera: antidiphtheritic sera (L. Martin), antimeningococcus (Nobécourt and Tissier), normal or physiological sera, and also to certain medicaments ordinarily well tolerated, such as iodide of potassium.

The greatest care therefore is required in estimating the rôle played by a serum in the temporary or definite improvement of a case under treatment. The sole criterion for judgement is *experimentation upon the tuberculous animal.* Yet this too is subject to error from many sources.

In attempting to treat patients by injections of serum more or less rich in antibodies, agglutinins, precipitins, etc., one is probably wasting his efforts; since, *however rich in these substances may be the best sera known today, in the doses in which they are injected they add almost nothing to the quantities of antibodies, agglutinins, etc., which the blood of the patient contains in much more considerable amount.*

Furthermore, we know that by introducing bacilli or bacillary

products (endotoxins, tuberculins, etc.) in progressively increasing doses into the normal or already tuberculous animal body, no one has as yet succeeded—although certain authors have claimed to—in producing in these animals true *antitoxins* (antituberculins) or *lysins* capable of dissolving the bacilli *in vitro* when protected by their ectoplasm of chitin, wax and fats. Hypervaccinated animals themselves are powerless to dissolve the bacilli in their own bodies; they preserve them in their lymph nodes for months or for years, inert but living and virulent, or else they eliminate them by all the normal paths for the excretion of cellular débris, principally with the biliary pigments (Calmette and Guérin).[32]

How is one to affirm that the injection into a tuberculous individual of sera derived from animals, which preserve the vaccinating bacilli or test bacilli intact in their bodies, can produce any bacteriolytic effects upon bacilli contained within the tuberculous cells?

Up to now, *therefore, it does not seem that specific serotherapy has realized the hopes which it aroused.*

[32] Ann. de l'Inst. Pasteur, 1911, **25**, 625.

CHAPTER XLII

ACTIVE IMMUNITY

Attempts at Vaccination with Toxins and Tubercle Bacilli

We have established through the facts stated in the preceding chapters, *that a tuberculous infection which has remained localized,* may confer upon the body *a peculiar state of intolerance toward new infections. We are dealing here with a form of immunity which finds expression in an aptitude for throwing off the bacilli like foreign bodies which the phagocytes and the digestive cellular juices do not succeed in causing to disappear.* This elimination is brought about *either through the normal paths of excretion* for the solid residues of the body fluids (bile passages, intestine and mucous excretions), *or through suppuration and necrosis of the tissues,* ending with the formation of cavities or of cold abscesses which finally open externally.

This conception of the mechanism of antituberculous immunity is of quite recent date. It was inspired by the study of the *phenomenon of Koch* and is the outcome of researches which I have carried out since 1905 with my collaborator C. Guérin and, subsequently, of those of certain other experimenters among whom should be cited principally Römer (of Marburg).

Some time before our work numerous attempts had been made to produce, in animals susceptible to bacillary infection, an immunity analogous to that which could be conferred against a certain number of bacterial diseases by the use of Pasteur's methods for the attenuation of virus, or the methods of vaccination by soluble poisons,—procedures resulting from the work of Roux and Yersin on diphtheria toxin.

These attempts, although most often fruitless, have at least taught us the forms of reaction of the tuberculosis-susceptible organism to bacilli of various origins and to the products derived therefrom.

It is worth while therefore to recall here the ideas which suggested them and to give the clinical or experimental results which may now be regarded as achieved.

A. ATTEMPTS AT VACCINATION WITH TUBERCULIN AND EXTRACTS OF TUBERCLE BACILLI

Tuberculous poisons (tuberculin or bacillary extracts), which have, so to speak, no toxic action upon organisms devoid of tuberculous infection, are incapable of conferring upon these organisms a true immunity. We have already seen that it is easy to accustom tuberculous animals to receive progressively increasing and even considerable doses of these poisons, without arresting the evolution of the tuberculous lesions. The evolution is merely retarded, and this, coupled with the preservation of a better general condition, is in itself of great benefit to the patient. But when administered as a preventive measure to healthy subjects who can tolerate enormous doses with impunity, the tuberculins do not confer any appreciable resistance. These facts stand out from the old experiments of Daremberg, Grancher and Hipp. Martin,[1] Babès,[2] Courmont and Dor,[3] Héricourt and Ch. Richet,[4] MacFadyean,[5] Pearson and Gilliland,[6] and others, upon guinea pigs, rabbits and dogs.

It is also clearly shown by the two following experiments, performed with my collaborator C. Guérin[7] in order to throw light upon this question:

A first heifer, proved to be tuberculosis-free by preliminary tuberculin tests, receives two inoculations of 10 cc. of raw tuberculin of Koch into the jugular vein, with a 10 day interval. The inoculations are well tolerated. Thirty days later she is tested with an intravenous inoculation of 3 mgms. of virulent bovine bacilli, a dose known to be fatal for control animals in about 28 to 35 days. During the following weeks the temperature is irregular, rising at times to 40°C. The animal emaciates and the general condition becomes bad. She is killed *in extremis* on the fifty-eighth day after the test. Her lungs are found full of tubercles, some translucent, the majority caseous. The bronchial and mediastinal glands, as

[1] Rev. de la tuberc., 1893, **1**, 289.

[2] Internat. Congr. on Tuberculosis, 1893.

[3] Internat. Congr. on Tuberculosis, 1891.

[4] Etudes expér. et clin. sur la tuberc., 1892, **3**, 365:—Bull. méd., 1892, 741: 906.

[5] J. Comp. Path. & Therap., 1901, **4**, 136; 1902, **5**, 60.

[6] J. Comp. Med. & Vet. Arch., (Phila.) 1902, Nov.

[7] Ann. de l'Inst. Pasteur, 1914, **28**, 329.

well as the liver, are equally involved. This heifer has therefore *had an acute rapid tuberculosis.*

A second animal, of the same age, receives as a preventive measure, under the same conditions, intravenously, first 0.20 gm., then 10 days later, 0.50 gm. of tuberculin purified by precipitation with alcohol. The test inoculation is also made 30 days later with 3 mgms. of the same virulent culture. The animal died on the fifty-seventh day with lesions as extensive as those of the preceding. These two experiments show that, in animals free from infection, preventive injections of raw or purified tuberculin, even in large doses, retard the development of the test infection only slightly, and are without immunizing power.

F. Klopstock[8] made identical observations in the guinea pig. He attempted to immunize this animal by injections of tuberculin in progressively increasing doses for several months. The guinea pigs thus prepared died from the test infection without there being any discoverable difference from the controls, either in the course of the tuberculosis or at autopsy.

Kapralik and Von Schrotter[9] tried to immunize human beings and also to treat patients by *inhalations* of tuberculin reduced to a vapor by very fine atomization. Thus they learned that an inhalation of about 30 mgms. of raw tuberculin was sufficient to produce a very strong reaction in cases of pulmonary tuberculosis. But the danger of this method is so great that one would not think of having recourse to it.

The administration of tuberculin *by the digestive tract* would not present the same objections. It was proposed in 1902 by Birnbaum in the form of keratin-coated pills, then in 1904 by Freymuth[10] who administered it in a dose of 10 to 80 mgms., after neutralization of the gastric juice with bicarbonate of soda. Krause has also employed it (tuberculin enclosed in keratin) in treatment and Latham and Imman noticed that it was thus capable of increasing the opsonic index in patients. But this mode of prevention and of curative treatment has been studied chiefly by B. Mollers and Heinemann.[11] From their researches these workers concluded that tuberculin is

[8] Ztschr. f. exper. Path. u. Therap., 1913, **13**, 56.
[9] Wien. klin. Wchnschr., 1904, **17**, 617.
[10] München. med. Wchnschr., 1905, **52**, 62.
[11] Veröffentl. d. R. Koch Stift. z. Bekämpf. d. Tuberk., 1912, H. 3, 29.

always more or less modified and weakened by the digestive juices, so that large doses, 1 gm. for example, at times produce no reaction effect. Repetition of these large doses does not lead to any process of immunization.

One cannot therefore think of utilizing the bacillary poisons (tuberculins or extracts) except to induce in already infected subjects *a greater resistance* to intoxication by these poisons, and therein, precisely, lies the aim of *tuberculin therapy*.

B. ATTEMPTS AT VACCINATION BY BACILLI KILLED OR MODIFIED BY VARIOUS PHYSICAL OR CHEMICAL AGENTS

1. Bacilli killed by heating

By repeating every 10 or 12 days the injection of very small doses of tubercle bacilli killed by boiling, I. Straus thought that he found a certain degree of tolerance in rabbits. Dembinski,[12] at Borrel's laboratory at the Pasteur institute, again took up the study of this question. After having observed that a normal rabbit died most frequently in 24 to 48 hours and at most in 28 days, after the intracerebral inoculation of 2 mgms. of powdered sterilized bacilli, he prepared several animals by injecting them intravenously every 10 days with 7 increasing doses: 0.00001 gm., 0.00002, 0.00005, 0.0001, 0.0002, 0.0005and 0.001 gm. All received afterward 2 mgms. intracerebrally. They showed themselves resistant, after having emaciated, but the majority of them developed caseous abscesses in the brain and meninges. Their resistance therefore was very limited.

Maragliano attempted to introduce for the prevention of human tuberculosis, a method of vaccination based upon the use of bacilli sterilized by heating. It consists in inserting a small amount of concentrated glycerinated bacillary suspension into a scarification made upon the arm, as for vacaination against small-pox. He inoculated a large number of persons in this manner, but the results, rigorously controlled at the *Henry Phipps Institute* at Philadelphia under the direction of Lawrence Flick, were found to be nil.[13]

Experiment shows that it is not possible to confer a lasting resistance upon a guinea pig by the repeated injection of dead bacilli subcutaneously or into the peritoneum. These injections always

[12] Compt. rend. Soc. de biol., 1903, **55**, 1409.

[13] Rep. Henry Phipps Inst., Philadelphia, 1904 *et seq.*

cause the formation of larger and larger sized abscesses or of peritoneal lesions which are finally fatal. I have already stated that Borrel had demonstrated this fact for the rabbit. But I succeeded, in collaboration with Maurice Breton,[14] in causing young guinea pigs to tolerate the ingestion of such doses of bacilli as were fatal for the controls in 35 to 80 days (5 mgms.). This we accomplished by making them ingest on two occasions at 45 days interval, 2 centigrams of bacilli which had been heated to 100°C. The test ingestion of virulent organisms took place 45 days after the second vaccinating ingestion. The immunity thus realized is however only relative and is not permanent, inasmuch as the animals die after 5 or 6 months with pleural, hepatic and occasionally pulmonary lesions.

Again we tried to vaccinate other young guinea pigs by causing them to ingest, with an interval of 45 days, first, 5 mgms. of bacilli heated to 100°C., and the second time 5 mgms. of bacilli heated to only 65°C. These animals were tested 45 days later, again by the ingestion of 1 centigram of virulent bovine bacilli. Some died considerably later than the controls, after 7 to 9 months. They presented discrete tuberculous lesions. Others, killed at the end of one year, were free from infection.

In cattle, bacilli killed by heat and injected intravenously are equally incapable of producing a state of lasting resistance, even if care is taken to alter the protoplasm as little as possible by heating to only 65°C. The following experiment, which I carried out with C. Guérin,[15] is proof of this:

A heifer from Brittany, aged 8 months, receives intravenously 20 mgms. of bovine bacilli, washed, then heated for 30 minutes at 60° on a water bath. Thirty days later the animal is tested by the intravenous inoculation of 3 mgms. of living bacilli, an amount constantly fatal for the controls. After a short period of high fever the general condition remains apparently satisfactory. Tested with tuberculin 90 days later, the heifer reacts violently (2.3°). It is slaughtered the same day. In the right lung are found seven caseous tubercles of the size of a millet seed and in the left lung four other tubercles, one of them as large as a grain of hemp and likewise caseous. The bronchial and mediastinal glands also contain numerous small caseated nodules.

[14] Ann. de l'Inst. Pasteur, 1907, **21**, 401.
[15] Ibid., 1914, **28**, 329.

Preliminary injection of heated bacilli had therefore simply modified the nature of the tuberculous infection which, instead of developing into an acute rapidly fatal miliary tuberculosis as is always the case with a dose of 3 mgms. in a normal animal became a *chronic slowly progressive tuberculosis.*

We obtained still less encouraging results in other cattle in substituting for the bacilli killed with heat, either bacilli of the same origin killed by simple aging of the culture, or two large doses of raw tuberculin of Koch (10 cc. each time with 10 days between), or of tuberculin purified by precipitation with alcohol (20 to 50 centigrams). The test was made intravenously 30 days after the second injection, with 3 mgms. of virulent bacilli. In all cases our animals suffered an *acute tuberculosis* and died only some 20 days later than the controls.

Experiments published later by Rothe and Bierbaum[16] led to the same conclusions.

Loeffler[17] believes nevertheless that at least partial immunity against tuberculous infection may be realized by means of bacilli first dried cold and then heated to 71° for 9 to 15 days. The capacity for resorption of the bacterial bodies within the animal organism is said to be considerably increased by this prolonged dry heating. Dogs, which are more susceptible to the human type bacillus than to the bovine type, may be immunized if injected first, intravenously or intraperitoneally, with bacilli thus killed with dry heat, afterward with doses of bovine bacilli increasing up to 100 mgms. They then tolerate the injection of 250 to 300 mgms. of human bacilli intravenously or into the peritoneum. With the sera (extremely rich in antibodies) of such dogs, therapeutic tests have been made in guinea pigs, but the results have not been favorable. Only liver tuberculosis appears to be influenced and the survival of the infected animals seems somewhat prolonged.

2. Bacillary lipoids and bacilli treated with lipoid solvents

The various lipoids which can be extracted from tubercle bacilli by treating them with appropriate solvents (see Chapter IV) do not possess, when employed after proper purification, any antigenic

[16] Veröffentl. d. R. Koch Stift. z. Bekämpf. d. Tuberk., 1913, H. 8/9, 138.
[17] Deutsch. med. Wchnschr., 1913, **39**, 1025.

property or immunizing power. This fact, brought out by the experiments of Wassermann and Citron, of Bruck, of Vallée,[18] of Seligmann and Pincus,[19] has been denied by Deycke and Much,[20] Kleinschmidt,[21] Dailmann,[22] Borissjack, Sieber and Metalnikov, and Kurt Meyer,[23] who claim to have obtained antibodies in animals into which they injected or caused to ingest either tuberculous waxes, or even pure lecithin in 10 per cent solution in olive oil.

In a series of experiments performed in collaboration with C. Guérin,[24] I ascertained that, in cattle at least, these lipoids are devoid of any vaccinating property. One of our animals, for example, which had received at 10 day intervals 100 and 200 mgms. of lipoids derived from bovine bacilli by extraction with boiling acetone, and then with benzine, developed an acute fatal miliary tuberculosis following the intravenous inoculation of 3 milligrams of virulent culture and was dead in 24 days.

On the other hand, the bacilli rid of lipoids by appropriate solvents which do not modify the protoplasmic substances too profoundly, can be utilized as antigens. According to the method of treatment, they give rise to a more or less abundant formation of antibodies (*see Chapter XXXVII*). They may be utilized for procuring amboceptor-rich sera in both animals and man, but efforts to use them as vaccines have been in vain. Nevertheless, J. Cantacuzène[25] states that he has succeeded in conferring upon guinea pigs a manifest resistance to the intoxication produced by fat-free bacilli. This he does by injecting the animals with bacillary bodies treated first with methyl alcohol, then with petroleum ether in a Soxhlet apparatus, and finally with Gram's iodin solution for 15 minutes.

Vallée also attempted to vaccinate calves with bacilli previously washed and dried, then rid of fats with petroleum ether by mechanically shaking for 50 hours in a flask containing glass beads. Bacteria thus treated had in large part lost their acid fastness. He inoculated

[18] Ann. de l'Inst. Pasteur, 1909, **23**, 585; 665.

[19] Ztschr. f. Immunitätsforsch., 1910, **5**, 377.

[20] Beitr. z. klin. d. Tuberk., 1910, **15**, 277.

[21] Berl. klin. Wchnschr., 1910, **47**, 57.

[22] Ztschr. f. Immunitätsforsch., 1911, **10**, 421.

[23] Ibid., 1912, **15**, 245.

[24] Ann. de l'Inst. Pasteur, 1914, **28**, 329.

[25] Compt. rend. Soc. de biol., 1905, **59**, 314; 316:—Ann. de l'Inst. Pasteur, 1905, **19**, 699.

25 to 100 mgms. into the veins. After a few days the injection was repeated without disturbance and the animals were said to have acquired a marked resistance to the effects of the intravenous inoculation of a dose of virulent bacilli which was rapidly fatal for the controls. In the horse, the inoculation of 100 to 150 mgms. of the same fat-free bacilli causes more or less serious trouble; but tolerance is developed rather quickly and, once obtained, the animal remains insensitive to intravenous inoculations of the various toxic products which can be extracted from the tubercle bacillus by maceration.

Louis Martin and Vaudremer[26] used to the same end a somewhat different method for removing the fats. They first wash the bacilli in ether in order to remove the water and glycerin, then dry them thoroughly and take them up once more with ether. In this the bacilli are allowed to remain for at least 6 weeks. Of the bacterial bodies so obtained, 5 centigrams are required to kill a normal guinea pig by intraperitoneal injection, while with the procedure of Vallée the fatal dose is 7 centigrams.

Guinea pigs which receive these fat-free bacilli intraperitoneally resist at times the inoculation of fatal doses of virulent bacteria, but the results are not constant.

3. Bacilli treated with various chemical reagents

Other investigators have attempted to treat the bacilli with chemical substances capable of destroying their vitality or merely attenuating their virulence.

With this end in view Moussu and Goupil[27] have tried chlorinated products. Bacilli macerated for more or less long periods in 10 per cent Javel water, for example, lose their acid fastness and disintegrate after three days. The product of this disintegration, centrifugalized, washed and neutralized, is inoculated into animals several times and at various intervals. In large doses it causes emaciation and death; in small amounts it confers a degree of resistance upon dogs and rabbits. In the guinea pig, according to my experiments with M. Breton,[28] the ingestion on two occasions of 1 centigram of chlorinated bacilli at 45 days interval, does not in any way vaccinate

[26] Compt. rend. Soc. de biol., 1906, **61**, 258.
[27] Compt. rend. Acad. des sci., 1907, **145**, 1231; 1359; 1908, **146**, 44.
[28] Ann. de l'Inst. Pasteur, 1907, **21**, 401.

against the effects of absorption of 1 centigram of virulent bacilli, also administered by the digestive tract.

Vallée's[29] attempts at prevention in young cattle were not much more successful. Into the jugular vein of these animals he injected 200 to 400 mgms. of bacilli macerated for one hour in 1 to 400 iodine water, or else had them ingest 20 to 50 centigrams of the same bacilli on two occasions 60 days apart.

The attempts on the part of Rappin[30] with sodium fluoride do not seem to have been more fortunate. His cattle vaccination experiments carried out in 1913–1914 before a Commission of the Société de Pathologie comparée, were unsuccessful. He was using dried bacilli, fluorated and sensitized.

H. Noguchi,[31] and later Marxner[32] tried to prepare a vaccine by treating bacilli with sodium or ammonium oleate, or with oleate of neurin, or again with oleic acid and soda alone. Deycke and Much[33] to the same end used *neurin* and *cholin*, Salimbeni,[34] *monochlorhydrin*. The results obtained do not seem to be appreciably better.

F. Levy, F. Blumenthal and Marxner,[35] instead of using reagents which profoundly modify the chemical constitution of the bacilli, tried to avoid this by treating the bacterial bodies with relatively harmless substances and they chose for this purpose glycerin, urea and galactose. The action of urea upon cultures of tubercle bacilli had been noted by Rappin[36] in 1901. The German authors treat tubercle bacilli (of which 0.0001 mgm. gives tuberculosis to the guinea pig) with a 25 per cent solution of urea for one day and thus obtain a culture so attenuated that the injection of 1 mgm. produces only a caseation of the glands near the point of inoculation, and that only after 2 or 3 months.

If the bacilli remain in the 25 per cent urea solution for two days, they are rendered completely harmless. On the contrary, a solution

[29] Ann. de l'Inst. Pasteur, 1909, **23**, 585; 665.

[30] Compt. rend. Soc. de biol., 1909, **66**, 410;—Compt. rend. Acad. des sci., 1909, **149**, 408; 1917, **164**, 421.

[31] Centralbl. f. Bakt., 1909, **52**, 85.

[32] Berl. tierarztl. Wehnschr., 1911, **27**, 115:—Ztschr. f. Immunitätsforsch., 1911, **10**, 118.

[33] Beitr. z. klin. d. Tuberk., 1910, **15**, 277.

[34] Compt. rend. Acad. des sci., 1912, **155**, 368.

[35] Centralbl. f. Bakt., 1906, **42**, 265; 1908, **46**, 278; **47**, 289.

[36] Compt. rend. Soc. de biol., 1901, **53**, 691.

containing 10 per cent of urea causes only a slight diminution of virulence.

The same phenomena are to be observed with bacilli macerated in 80 per cent glycerin or in 25 per cent galactose.

Four to five days in the solution of galactose, glycerin or urea, with frequent shakings, suffice to kill 5 milligrams of bacilli. The latter then become innocuous. They may be employed either as vaccines capable of conferring a slight resistance to tuberculous infection,—at least in the guinea pig,—or as a therapeutic agent. This is the *Tébéan*, put on the market by the firm of *E. Schering* (of Berlin) for the tuberculin therapy of tuberculosis.

Deycke and Much[37] state that they have obtained very encouraging results by injecting tubercle bacilli which have been suspended in contact with ovolecithin. Of 27 animals thus treated preventively, 10 were completely immunized, 8 but partially and the other 9 not at all. Lindemann,[38] in the laboratories of the K. K. Gesundheitsamte of Berlin repeated their experiments. He treated many guinea pigs with suspensions of bacilli which had been macerated in the incubator in lecithin for one, two and up to four weeks. In no case was immunity obtained.

L. Rabinowitsch[39] prefers not to kill the bacilli and tries to modify them by growing them in the presence of small quantities of formalin. The virulence is attenuated under these conditions, and guinea pigs which receive 2 mgms. of these bacilli subcutaneously resist, some weeks later, the inoculation of a dose rapidly fatal for the controls.

It would seem that the weak acids, notably lactic acid, according to Deycke and Much,[40] produce analogous effects. They cause all the protoplasmic substances that take the *Gram* stain to disappear at the end of a certain time, and also the acidophile components. By centrifugating, products are obtained, both in the sediment and supernatant fluid, which separately may fill the rôle of antigens (partial) and to which special antibodies are supposed to correspond. Immunity against tuberculosis, according to these authors, is due to the combined action of these various antibodies.

[37] München. med. Wchnschr., 1909, **56**, 1985:—Centralbl. f. Bakt., 1910, **54**, 342.

[38] Centralbl. f. Bakt., 1914, **74**, 624.

[39] Berl. klin. Wchnschr., 1913, **50**, 114.

[40] München. med. Wchnschr., 1913, **60**, 119; 190.

Passini and Wittgenstein[41] had the idea of liquefying tuberculous sputum by leaving it to itself, at a temperature of 37 to 40°C., under a layer of toluol and under an increasing atmospheric pressure. By filtering afterward through porous porcelain or infusorial earth, they obtain a clear liquid which, injected subcutaneously into patients even in large doses, produces no harmful effect and would seem, on the contrary, to favorably influence the evolution of the disease. A local reaction is observed at the point of inoculation and, if the quantity of filtrate injected is considerable, a temperature reaction may appear. The latter is said to be followed by a quite evident improvement in the general condition.

The authors of this procedure consider that they have realized the ideal *auto-tuberculin vaccination, combatting* at one and the same time *the tubercle bacillus* and *the organisms of the mixed infection.* The clinical results obtained up to now do not, however, seem to justify their hopes.

Finally, F. Loeffler[42] attempted recently to vaccinate animals with tubercle bacilli digested in part with *Carnevorin* (extract of the juice of the *Drosera*). This product diluted 1 in 3, kills bacilli of the human type in 48 hours and those of the bovine type in 72 hours. But the bacilli thus treated do not have any immunizing action.

4. Bacilli killed or modified by light rays

Instead of employing chemical reagents, whose effects upon the protoplasm are always more or less severe, various workers have attempted to abolish or attenuate the virulence of tubercle bacilli with various physical agents. In this way Di Donna tried to utilize as a vaccine bacilli killed by exposure to sunlight. V. and Mme. Henry tried ultra-violet rays. But the various attempts of this sort have had no practical result.

Unfortunately the use of the diastases does not seem to be giving us anything more than interesting information. There are the diastases foreign to the animal organism, for example, papaine or the vegetable pepsins such as the juice of the *Drosera* which, as said above, has been studied by Loeffler as to its effects upon the tubercle bacillus. There are also certain cellular ferments (protease, lipase,

[41] Wien. klin. Wchnschr., 1911, **24**, 1083.

[42] Deutsch. med. Wchnschr., 1913, **39**, 1025.

etc.), for example, those which are produced by the lymphocytes of the glands or of the spleen, to which Livierato, J. Bartel and later Fontès attributed modifying properties in the sense of attenuating virulence. These properties are such, according to them, that virulent bacilli, left for a long time in the juice of lymphatic glands, *in vitro* or *in vivo* might be employed as vaccines. We shall speak of them again very soon.

In brief, none of the methods aiming at a more or less complete destruction, by physical or chemical agents, of the vitality of tubercle bacilli in order to transform the latter into vaccine, have yet given satisfactory results. From the experiments along this line one has the impression nevertheless that the methods which do not alter the bacillary protoplasm too profoundly may have certain favorable effects upon resistance to severe infections. And if we bring these facts into relation with what we have learned of the protective influence exerted by a preëxisting slight in fection upon a subject already tuberculous, as regards *superinfections*, it is natural to think that antituberculous vaccination will have more chance of being effective if based upon the utilization of bacilli *still alive, but deprived as far as possible of their ability to induce follicular lesions.*

C. ATTEMPTS AT VACCINATION WITH VIRULENT AND ATTENUATED BACILLI

The first attempt at immunization with living bacilli appears to have been made in 1886 by Cavagnis with progressively increasing doses of tuberculous sputum to which carbolated water had been added. Somewhat later, in 1889, Grancher and Ledoux-Lebard sought to vaccinate rabbits against avian tuberculosis by the same procedure of increasing doses, but their results were practically negative. Then Grancher and Hipp. Martin[43] (1890) thought to obtain better results by inoculating, either intravenously or subcutaneously, cultures much attenuated by aging on artificial media, then afterwards cultures younger and younger and more and more virulent. The rabbits thus treated were more resistant than the controls, but they did not survive long and almost all of them finally died with kidney lesions of the *epithelial glomerulo-nephritis* type.

[43] Rev. de la tuberc. 893, 1, 289.

Richet and Héricourt,[44] Gilbert and Roger, Courmont and Dor, Dixon, Trudeau, and Von Schweinitz, made other similar attempts on the dog, or on the guinea pig, using avian or human tubercle bacilli. Their success was no greater.

1. Bovovaccination of Behring

The really interesting and fruitful period of experimental vaccination of larger animals begins with von Behring[45] who, in 1902, with Römer[46] and Ruppel, made known the method which he termed,—incorrectly,—*jennerisation* of cattle.

It consists in inoculating young calves intravenously on two occasions with a small quantity (4 milligrams at first, then 20 milligrams weighed dry) of a culture of human tubercle bacilli, maintained for six and a half years in the laboratory, then dried *in vacuo*, and whose virulence for the guinea pig is extremely reduced. The interval between inoculations was first fixed at six weeks and later at three months. It is essential that the animals be kept away from any possible accidental infection, by isolating them in a special stable during the time necessary for the immunization and during the six weeks thereafter.

The method was widely studied experimentally and important applications were made to cattle, particularly in Germany, Hungary, Denmark, Sweden, Italy, France and the United States. On July 13, 1904, the *Société de médecine vétérinaire pratique*, on the proposition of the elder Rossignol, decided to undertake test experiments at Melun on a sufficiently large scale to judge of its value.

They were begun in December 1904 and were to determine:

1. As to the harmlessness of the vaccination;
2. As to whether it was effective;
3. The duration of the immunity conferred upon animals of the bovine species.

The experimental test was carried out upon 21 animals aged from 6 months to 1 year. They had been previously tested with tuberculin and stabled in a disinfected place which had never been used for cattle.

[44] Compt. rend. Soc. de biol., 1894, **46**, 152.
[45] Tuberk. Beitr. z. exper. Therapie, 1902, H. 5; H. 8.
[46] Ibid., 1902, H. 7.

The 21 calves were vaccinated twice with a 90-day interval, December 11 and March 12, 1904. One of them died 57 days after the first vaccination. Another was slaughtered 9 months after the second vaccination.

These two animals were found free from tuberculosis and their bronchial glands were not infectious for the guinea pig.

It was possible therefore to conclude that *the vaccination is harmless.*

The 19 vaccinated animals remaining were divided into four lots:

a. Six *vaccinated* animals and 6 *normal controls* were tested by intravenous inoculation of 4.5 milligrams of *virulent bovine bacilli.*

b. Two *vaccinated* animals and 2 *normal controls* were tested by *being made to live at close quarters* with cattle having open pulmonary tuberculosis.

c. Seven *vaccinated* animals and 7 *controls* were tested by *subcutaneous virulent inoculation.*

d. Four *vaccinated* animals were protected from infection, with a view to future tests.

Of the 6 vaccinated animals of lot *a*, *tested intravenously*, 4 were found on autopsy to be free from tuberculosis, but *their bronchial glands were infectious for the guinea pig.* Two showed very slight lesions of the glands connected with the lungs. Of the 6 controls inoculated in the same manner, 3 died in 29, 34 and 37 days; the other 3 were slaughtered and showed massive lesions of generalized tuberculosis.

Of the two vaccinated animals of lot *b*, *tested by being stabled with infectious animals* for one year and then slaughtered, one showed extensive lesions of tuberculosis, the other merely a small focus in the left tonsil; while in the two controls, slaughtered after 6 months of stabling with infectious animals, very extensive lesions were found at autopsy.

Of the 7 vaccinated animals of lot *c*, tested subcutaneously, 4 presented at autopsy fairly mild local lesions, accompanied, in the case of 3, by very slight lesions of the prescapular gland. The three others had no local lesions, but their prescapular glands were found infectious for the guinea pig. The 7 controls had serious local lesions as well as extensive pulmonary and glandular lesions.

The 4 animals of lot *d* were reserved for computing the duration of the immunity. Two of them were tested intravenously one year after the second vaccination; one died in 47 days and the other, although remaining apparently in good condition was found after one year to have generalized tuberculous lesions.

The two others were tested by being stabled with infectious animals, two years after the second vaccination.

After a 33-day contact with a calf affected with tuberculous pneumonia, a single animal contracted a fairly extensive tuberculosis of all the glands connected with the lung.

The report presented by Vallée and Rossignol, on October 31, 1906, in the name of the *Société de médecine vétérinaire pratique*, drew from the test experiments the following conclusions:

1. The quite definite resistance offered by the vaccinated animals to intravenous infection 3 months after the bovovaccination, exhausts itself fairly quickly.

2. The resistance of the vaccinated subjects against contagion, such as results from contact in stables with animals having open lesions of tuberculosis, is not very marked and does not last beyond a few months.

On the other hand it was found, in supplementary experiments made by the Commission, that the *bovovaccine* furnished by von Behring's laboratory at Marburg is not of uniform virulence for the guinea pig. Certain samples produce tuberculosis in these animals, whereas others are innocuous. It is therefore permissible to suppose that the effects of this vaccine in cattle are not always identical.

Perhaps in these inequalities of virulence is to be found the principal reason for the difference in results obtained by many who have published their tests. The reports of Hutyra,[47] of Lorenz,[48] of Thomassen,[49] of Pearson and Gilliland,[50] of Belfanti and P. Stazzi,[51] of Degive, Stubbe, Liénaux and Mullie,[52] of Theobald Smith,[53] of G. Regner and O. Stenström,[54] are particularly instructive on this subject.

[47] Internat. Congr. on Veterin. Med., 8th, Budapest, 1905.

[48] Ibid.

[49] Ibid.

[50] Penn. Univ., Dept. Med., Med. Bull., 1906;—Vet. J., Lond., 1907, 101; 139.

[51] Clin. Veter., 1906, **29**, 313.

[52] Ann. de méd. vetér., 1906, p. 76.

[53] J. Am. Med. Assn., 1906, **46**, 1247;—J. Med. Research, 1908, **18**, 451.

[54] Centralbl. f. Bakt., 1908/09, **48**, 628; 1913, **72**, 180.

Eber[55] (of the *Imperial Health Office* at Berlin) reported observations made upon two large herds. Cattle were portioned out among 8 farms in which from 43.8 to 100 per cent of the animals were infected.

There were 213 cattle bovovaccinated from 1904 to 1906. Ten died during the 3 months following the first vaccination; 6 were autopsied; none were found tuberculous.

At the end of 1906 and at the beginning of 1907, 148 of the vaccinated animals were tested with tuberculin with the following results:

Of 70 animals aged from 6 to 18 months	19 or 27.1 per cent reacted
Of 49 animals aged from 18 months to 2 years	22 or 44.9 per cent reacted
Of 26 animals aged from 2 years to 3½ years	15 or 57.7 per cent reacted
Of 3 animals aged from 3½ to 4½ years	0 or 0.0 per cent reacted

To sum up, the vaccination had no influence upon the development of the disease.

Of 19 vaccinated cattle which were slaughtered, 9 were found tuberculous; 5 had generalized lesions. The lesions were twice found localized in the bronchial nodes, once in the lungs, and once in the mesenteric glands.

From his own findings and from an analysis of documents published from various sources after 10 years trial, Eber[56] draws the following conclusions:

1. The resistance of young cattle to experimental infection can be notably increased by the use of tuberculous virus from various sources. This resistance is never absolute. The vaccinated animals become infected if large doses of virus are used.

2. The maximum increase of resistance is to be found the most certainly some time after vaccination (3 months on the average).

It cannot be confidently stated whether this period is preceded by a lowering of resistance.

3. Artificial increase of resistance is not of long duration.

4. The finding of a manifestly increased resistance to experimental infection with virulent material does not imply that the same animals would not behave in an entirely different manner in the presence of natural contagion (stable contagion).

It is therefore perfectly well established that the bovovaccine of

[55] Deutsch. tierarztl. Wchnschr., 1907, **15**, 557; 573.

[56] Centralbl. f. Bakt., 1907, **44**, 463; 569; 1916, **78**, 321.

von Behring confers upon cattle an appreciable resistance to various modes of natural or artificial infection; but this resistance,—of short duration inasmuch as it hardly exceeds 12 to 14 months,—manifested in so far as it persists, by a relatively complete absence of tuberculous lesions, is not sufficient to enable the organism to resorb the virulent test bacilli, nor even those introduced as vaccines.

Many of them are retained, at least in part, for months, in the glands (tracheo-bronchial and mediastinal principally) and remain there, ready to announce their presence more or less abruptly by anatomical disturbances when the resistance artificially conferred by the vaccine injection is about to give way (Hutyra, Thomassen,[57] Baumgarten,[58] and others). In a recent work, Baumgarten states that he has obtained favorable results with von Behring's vaccine by injecting human bacilli, not intravenously, but subcutaneously into cattle, in doses of 5 centigrams the first time, then 3, 2 and 1 centigrams for the other treatments. Thus he has seen cattle resist 7 tests with virulent bovine bacilli inoculated over a period of 4 years. Of 48 bullocks so treated, 11 are said to have been definitely immunized.

The *Massachusetts Commission* reported that calves, after inoculation with human tubercle bacilli, succumb at times to a special type of tuberculous pneumonia which is never observed to occur spontaneously. This disease may lead to death in one or two months. It may also be complicated with tuberculous localizations in the eyes, from which complete blindness results.

The animals vaccinated with human bacilli remain very susceptible to tuberculin for 8 to 12 months.

The *bovovaccine* prepared under the control of von Behring's laboratory at Marburg, is delivered to veterinarians in tubes of 5 and 20 units (1 unit corresponds to 4 mgms. of bacilli). Each tube contains respectively 2 and 10 cc. of bacillary emulsion to be injected intravenously at an interval of 12 weeks.[59] The vaccine preserves its activity for 30 days, but beyond this period, its efficacy diminishes.

[57] Rec. de méd. vét., 1903, 8. s., **10**, 5.

[58] Berl. klin. Wchnschr., 1904, **41**, 1124:—Beitr. z. path. Anat. u allgem. Path., 1917, **63**, 259.

[59] Römer (Kraus and Levaditi: Handb. d. Tech. u. Methode d. Immunitätsforsch., Ergänzungsbd., I, 1911, p. 327) proposed a single inoculation of 5 units (20 mgms.) intravenously; to be repeated after one year. This method does not seem to offer any appreciable advantage over the first and as yet no proof of its practical value has been offered.

Since it is made up of human bacilli, although the virulence of the latter is attenuated by the aging of the cultures, the operator and his assistants should be very careful in the course of the manipulations not to contaminate themselves and not to spread the contamination round about the vaccinated animals.

In fact, it is established that these animals eliminate tubercle bacilli intermittently for a long time, in their dejections and particularly through the mammary glands, and that the bacilli retain the characters of the human type. For milch cows particularly, or rather for those who drink their milk, this elimination may offer grave danger.

A. Stanley Griffith[60] told of one cow which had received an intravenous injection of 0.15 gm. of a culture of human tubercle bacilli and which, 5 months and a half later, showed tubercle bacilli in pus from the teats. By the 8th month, a tuberculous mastitis appeared and, 549 days after the vaccination, tubercle bacilli could still be disclosed, their human origin indicated by cultural characters and by their attenuated virulence for the rabbit. In another case, the milk contained tubercle bacilli 155 days after an injection of 0.10 gm. of the human culture intravenously.

Although the fear of a such a survival of bacilli has generally prevented vaccination of milch cows, it was regarded as at least altogether improbable that the bacilli would continue to live in adult animals which had been vaccinated in quite early life. Yet Griffith examined the milk of two cows which had been vaccinated 4 days after birth, and was able to recover human bacilli.

The imperfect results obtained by the method of v. Behring have caused many investigators to seek modifications which might increase its effectiveness or obviate its objectionable features. The seriousness of the latter, after what has just been said, makes extreme caution imperative.

2. Tauruman of R. Koch, and Schütz, Neufeld and Miessner

Robert Koch, Schütz, Neufeld and Miessner,[61] in 1905, reported on certain attempts which they had been making since 1902 to immunize cattle with attenuated human and bovine bacilli. Certain

[60] J. Path. & Bact., 1913, **17**, 323.

[61] Deutsch. med. Wchnschr., 1904, **30**, 660; 1241:—Ztschr. f. Hyg., 1905, **51**, 300.

types of human bacilli are particularly suited to this end. According to Neufeld, the ass and the goat can with certainty be immunized against large doses of very virulent bovine bacilli. A single intravenous injection of 1 to 3 centigrams of human bacilli from a 30 to 40 days old culture on glycerin broth, blotted with paper, then dried and suspended in 10 cc. of physiological salt solution, protects against an intravenous inoculation of 2 centigrams of bovine bacilli, made 103 days later. The same result is obtained moreover with an attenuated bovine bacillus, injected also into the jugular vein, in a single dose of 1 to 3 centigrams. Koch and Schütz made a vaccine from them which, in Germany, is delivered to veterinarians in the form of an emulsion and to which they have given the name of *Tauruman* (prepared by Lucius, Meister and Company of Höchst, near Frankfurt on the Main).

This vaccine, while having no real advantages, has features more objectionable than that of von Behring, since it with still more certainty produces the persistent bacillary infection of the lymphatic glands. Weber and Titze, and Schütz and Holland have acknowledged that *one month after an inoculation of Tauruman, all of the organs of the vaccinated animals are virulent for the guinea pig.*

Moreover, C. Titze[62] found that the bacilli of *Tauruman* inoculated intravenously into cows, might be excreted in the milk. In one case he recovered some at the 3rd week, and the milk still contained them on the one hundred and forty-fourth day. In the second case, the bacilli were excreted by a single teat. In still another case, the excretion was prolonged for 16 months.

Furthermore, the experiments performed by Weber and Titze[63] at the *Imperial Health Office* at Berlin, are not very favorable to the practical use of Tauruman. In addition to the fact that the bacilli composing it are virulent for the guinea pig, the cattle which were inoculated at the age of 3 weeks had lost all resistance after 9 months. Others, tested by inhalation after 5 and 8 months, and slaughtered on the two hundred and seventy-second day, presented tuberculous lesions in the bronchial and mediastinal glands. The vaccinated animals infected through living with other animals showed themselves as a rule but little resistant. Those vaccinated through ingestion were more so, at least up to 8 months.

[62] Tuberk.-Arb. a. d. k. Gsndhtsamte, 1908, H. 9, 50.
[63] Ibid., 1907, H. 7, 1.

Inoculations of *Tauruman* seem, in all cases, to dull the sensitiveness to tuberculin: the reaction most often becomes doubtful. On the other hand many young calves contract a fatal pneumonia (pneumonia of young calves) following the vaccination.

Baumgarten, and later Lignières proposed to introduce the vaccine subcutaneously instead of intravenously, in order to localize the infection of the tissues by the virulent bacilli; but it seems indeed, from trials by S. Arloing, Pearson, Weber and Titze, and Vallée, that the results are then worse.

3. Method of Heymans

Heymans[64] (of Ghent) thought that he succeeded in vaccinating cattle by inserting into the subcutaneous connective tissue a permeable sac (of reed pith) containing human tubercle bacilli, properly selected and modified. The Belgian Government named a Commission to test out the method on a large scale, from March 1908 to the end of 1911. The report of this Commission, published in the *Bulletin du Service de Police sanitaire des animaux domestiques* (April 16–30, 1912) was definitely unfavorable. It concluded that Heymans' sac did not vaccinate animals submitted to the test of being stabled with other animals or of ingestion, nor animals exposed to ordinary contagion on farms, and that the method is without practical value.

4. The method of Klimmer

Following the publications of Friedmann[65] and of Moeller,[66] there was some hope for a moment in the use of bovine or human bacilli modified by successive passages through the body of cold-blooded animals (*turtle, crocodile, slow-worm, carp, salamander*). But before long it was shown, on the one hand, that human and bovine bacilli do not multiply in the organism of cold-blooded animals and that, when withdrawn from them, their virulence remains the same; on the other hand, that the tubercle bacilli peculiar to these cold-blooded

[64] Bull. Acad. roy. de méd. de Belg., 1904, 4. s., **18**, 319; 780:—Arch. internat. de pharmacodyn., 1906/07, **16**, 245; **17**, 113; 1908/09, **18**, 179; **19**, 337; 1910/11, **20**, 147; 1912/13, **22**, 243; **23**, 299; 1914/18; **24**, 55.

[65] Deutsch. med. Wchnschr., 1903, **29**, 953; 1904, **30**, 166.

[66] Ztschr. f. Tuberk., 1903, **5**, 206.

creatures are devoid of all vaccinating capacity for mammals (Dieudonné,[67] Weber and Taute[68]).

Klimmer[69] (of Dresden) meanwhile proposed the use of a mixed vaccine, called *antiphymatol* and prepared, under his direction by the House of *Hermann and Teisler*, of Donah:

(1) With human tubercle bacilli attenuated by heating at 52 to 53°C., said to be no longer pathogenic for the guinea pig (*vaccine* TH); (2) With human bacilli which he had accustomed to live in the body of salamanders through successive passages, which were said to be no longer pathogenic for any mammals, and which he asserted were no longer the ordinary acid-fasts (*vaccine* AT).

The inoculation of these two types of vaccine into the jugular vein never caused any accident. Yet the author prefers to recommend the subcutaneous injection. He has not observed any local or general reactions following the inoculation of either normal animals or those mildly tuberculous. Resistance is acquired after two months and begins to diminish at the end of one year.

This preparation, which was tried out particularly in Saxony on a certain number of cattle, with unconvincing results (Edelmann),[70] does not seem any more efficacious than the *bovovaccination* of von Behring. According to Krautstumk[71] and also to Eber,[72] it is not to be recommended.

5. *Vaccine of S. Arloing*

We know that S. Arloing[73] succeeded in causing tubercle bacilli from different sources to vegetate in the depth of broth cultures, by shaking the latter either continuously or intermittently. Certain human bacilli maintained under these conditions, at a temperature of 37°C. gradually elevated to 44 and even 46°C., and frequently replanted, acquired a special and fixed virulence. When injected subcutaneously or intravenously, or ingested in suitable dosage, they cause follicular lesions only rarely and they produce, in the small animals, an infection which is usually benign and of a septicemic form. In large doses they give rise to tubercles.

[67] München. med. Wchnschr., 1903, **50**, 2282.
[68] Tuberk.-Arb. a. d. k. Gsndhtsamte, 1905, H. 3, 110.
[69] Ztschr. f. Thiermed., 1908, **12**, 81.
[70] Ber. u. d. veterinärwesen im K. Sachsen, 1909, p. 216.
[71] Ztschr. f. Infektionskr. . . . u. Hyg. d. Haustiere, 1913, **14**, 366.
[72] Centralbl. f. Bakt., 1916, **78**, 321.
[73] Compt. rend. Acad. des sci., 1906, **142**, 1395; 1487; 1909, **149**, 962.

The tolerance of cattle to this organism is generally very great, so that it might serve as a vaccine.

This vaccine has been used by S. Arloing, and later by his son F. Arloing,[74] not only in laboratory experiments, but also on several large farms. S. Arloing thinks that it is without danger for the operator, although he has not proved it. He administers it in two separate intravenous injections with an interval of 3 to $3\frac{1}{2}$ months, and only in cattle previously tested with tuberculin and recognized as free from infection.

The vaccination does not give rise to any accidents. As to practical results, it is hard to judge, since up to now no test has been carried out to determine how much resistance the vaccinated animals possess to prolonged infection through close stabling. We are also ignorant as to the duration of the immunity.

S. Arloing has observed that after a test inoculation of bovine virus, 50 per cent of his vaccinated animals showed no lesion at autopsy; 25 per cent had circumscribed glandular lesions and 25 per cent had lesions disseminated as in the controls.

On a farm near Bourges (Cher), about 77 per cent of the vaccinated cattle are said to have resisted infection in the midst of other cattle of which 81 per cent were tuberculous.

The vaccinated animals retain the faculty of reacting to tuberculin for a long time, perhaps for more than one year. It is probable that when they cease to react, they have lost the resistance to tuberculous infection which the vaccination had conferred upon them. But this question had not yet been settled when the death of S. Arloing unfortunately intervened.

6. Method of Theobald Smith

Following the experiments made with the *bovovaccine* of von Behring by the *Commission of the Society of Agriculture of Massachusetts,* Th. Smith[75] was led, by reason of breeding conditions peculiar to the United States, to study a simplified method of vaccination based upon a single intravenous injection of a suitable dose of a culture of bovine bacilli attenuated by aging. The culture is nevertheless still quite virulent since it kills young cattle in a dose of 10 mgms. It may be

[74] J. de méd. vét. et zootech., 1913, 5. s., **17,** 577.
[75] J. Med. Research, 1908, **18,** 451; 1911, **25,** 1.

employed as vaccine in a dose of 1 to 2 mgms., but the animals must afterward be isolated for several weeks. This procedure, by reason of its dangers, has not been used practically.

7. Vaccination of cattle with the avian bacillus

MacFadyean, Sheather, Edwards and Minett[76] have tried the intravenous inoculation of avian bacilli as a method of immunization against bovine tuberculosis. They used, parallel with other attempts with the human bacillus, doses of 8 to 50 mgms. of avian culture in two injections with a 45-day interval. With very rare exceptions, inoculations had no harmful effect upon the subjects. The animals were later injected subcutaneously with the test virus. The glandular and visceral lesions observed were less serious in the animals vaccinated with the human bacillus than in those vaccinated with the avian bacillus; but autopsy of the controls showed that even with the avian bacillus the resistance conferred is considerable. MacFadyean and his collaborators feel that when it is thought necessary to vaccinate young animals, recourse should be had rather to the avian bacillus than to the human bacillus, in order to avoid transmitting the disease to man through the bacilli which remain alive in the body of the vaccinated animals and are eliminated with the milk.

8. Method of Friedmann

Friedmann[77] has isolated, from a water turtle, an acid-fast bacillus which is devoid of virulence for warm-blooded animals. He has proposed its use not only as a vaccine against tuberculosis of cattle, but also as a curative agent in human tuberculosis.

Orth experimented with this bacillus upon the guinea pig. He observed that it could live for months and even for years in the body of this animal, at times inducing tuberculous lesions which were typical, but attenuated and compatible with health. The animals so inoculated are said to have acquired some resistance to bovine and human strains. They survive longer than the controls.

These results indicate that Friedmann's cultures are not made up solely of acid-fast bacilli from the turtle, but that they also contain human bacilli. We know in fact from the experiments of Weber

[76] J. Comp. Path. & Therap., 1913, **26**, 327.
[77] Deutsch. med. Wchnschr., 1903, **29**, 953; 1904, **30**, 166; 1673.

and Titze, and from those of other workers, that the bacilli of cold-blooded animals have no vaccinating power against the bacilli of warm-blooded animals.

Moreover, the claims of Friedmann, as to the curative action of his organism in human tuberculosis, have been tested out by clinicians in Germany and in the United States and found to be unsound (J. Israel, Wolff-Eisner, Rautenberg, Bohm, F. Klemperer, F. Meyer, Ernst W. Frank, Borchardt, Mühsam, H. L. Barnes,[78] and others). Several believe indeed that certain patients whom they injected exactly according to the indications of Friedmann, became considerably worse (Brauer,[79] G. Mannheimer,[80] and others).

9. Vaccine of J. Ferran

For several years J. Ferran (of Barcelona)[81] has been carrying on experiments which, in his opinion, go to show that the tubercle bacillus is derived by a series of successive "mutations" from a saprophytic non-acid-fast bacterium incapable by itself of producing tuberculous lesions, but able to generate inflammatory lesions.

This original microörganism is the bacterium α. The bacterium β, derived from the preceding by successive passages through the guinea pig, already less easily cultivated, produces toxins analogous to lipoids in their nature and which act locally in the manner of tuberculous fats. The bacterium δ is the typical bacillus of Koch. The bacterium γ is said to be a return to previous forms by atavistic mutation: it has lost its acid-fastness, grows in masses and no longer has the characteristic odor of cultures of true tubercle bacilli.

The ubiquity of the bacterium α should explain the wide diffusion of tuberculous infection, in particular the frequency of positive reactions to tuberculin in healthy persons. Its harmlessness should permit of its being used as a vaccinating virus.

I was able to perform a few experiments in collaboration with L. Massol,[82] starting with cultures which J. Ferran had been good enough to send me. I must say that we could not obtain the above

[78] Providence Med. J., 1913, **14**, 254.

[79] Hamburg med. Uberseeh., 1914, **1**, 387.

[80] Ztschr. f. Tuberk., 1914, **22**, 560.

[81] *Traveaux sur la nouvelle bactériologie de la tuberculose.* Barcelona 1913. La Renaixensa.

[82] Compt. rend. Soc. de biol., 1913, **74**, 21.

results in following out the prescribed technique. The guinea pigs which had received repeated injections of atoxic tuberculogenic bacteria, non-acid-fast, did not react to tuberculin. Their sera furnished antibodies corresponding to these bacteria, but did not give the complement fixation reaction with tuberculous antigens.

The experiments of J. Ferran would have to be repeated by several investigators in order to establish whether the *mutations* may actually occur as the eminent scientist of Barcelona believes. At the present time we cannot regard this important question as settled.

10. Vaccination with sensitized tubercle bacilli

Vallée and L. Guinard[83] attempted to apply to tuberculosis the method of preparation of sensitized bacilli as devised by Besredka. For that purpose they made use of a horse serum prepared by Vallée (of Alfort), which gives an abundant precipitate in the presence of raw tuberculin of *Koch* or of various bacillary extracts. This precipitate, they thought, must be made up of tuberculin modified by contact with serum, since it is practically harmless for tuberculous guinea pigs, and since considerable doses can be given intravenously to infected cattle without harm and without producing a tuberculin reaction. But it is now known, from experiments which I published with L. Massol,[84] that this precipitate in reality contains no trace of tuberculin; the latter remains intact in the supernatant fluid after centrifugation.

Fritz Meyer[85] treated his bacilli directly with the antituberculous serum of Ruppel and Rickmann, prepared by *Hoechst* near Frankfurt on the Main. This serum is highly agglutinating and contains a large amount of antibodies.

The bacilli thus sensitized are said to be about 5 times less toxic for tuberculous guinea pigs than the same bacilli non-sensitized. They are supposed to confer upon normal animals a resistance to tuberculosis such as would cause the infection to develop about 6 to 8 times more slowly than in the controls.

Their absorption subcutaneously is said to be very rapid, even in man.

[83] Compt. rend. Acad. des sci., 1910, **150**, 1140.

[84] Ibid., 1910, **151**, 285.

[85] Berl. klin. Wchnschr., 1910, **47**, 926.

F. Meyer has introduced these bacilli into tuberculosis therapy. The patients receive progressively increasing doses subcutaneously. We lack sufficient experimental knowledge to be able to judge of the value of this method. The numerous attempts at sensitization of tubercle bacilli which I have been able to make with various sera, particularly rich in antibodies, have always given entirely negative results. Not only did the bacterial elements fail to absorb, even after infection by simple instillation into the eye, but the animals often became more quickly and more seriously tuberculous than did the controls.

11. Attempts at attenuation of the virulence of tubercle bacilli in the digestive tube of the leech

F. Marino[86] observed that tubercle bacilli introduced into the digestive tract of leeches remain for a long time intact. They are recovered after 15 or 16 months and have then lost the greater part of their virulence for the guinea pig. By cultivating these bacilli in glycerin broth, allowing them to be again ingested by leeches, and by thus repeating the passages several times, Marino succeeded in obtaining a strain which lived for 5 years in this cycle: leech—guinea pig—broth. It is this strain which he proposes to use as a vaccine, but up to the present there is no experimental work enabling us to evaluate its efficacy.

12. Attempts at vaccination with emulsions of tuberculous glands

A. Rodet and Garnier[87] first had the idea, in 1903, of finding out whether certain organs of tuberculous animals, especially the lymphatic glands, have more preventive properties against experimental tuberculosis than do the bacilli themselves or their products isolated from cultures. In their experiments they made use of non-caseated guinea pig lymph nodes, making emulsions which they preserved for several days by the addition of thymol in order to assure the death of the bacilli. The results obtained were completely negative. Later Livierato, then Bartel and Neumann,[88] afterward Manfredi and Frisco,[89] again undertook the study of this question.

[86] Compt. rend. Soc. de biol., 1911, **71**, 220.

[87] Ibid., 1903, **55**, 1109; 1111; 1112.

[88] Centralbl. f. Bakt., 1908/09, **48**, 657:—Wien. klin. Wchnschr., 1907, **20**, 1321; 1364.

[89] Centralbl. f. Bakt., 1902/03, Ref., **32**, 295.

According to these experimenters, the lymphatic glands not only retain the tubercle bacilli as does a filter, but they exert some sort of specific action upon the bacilli modifying their virulence.

Bartel[90] attributes this property above all to the lymphocytes and chiefly to the mesenteric glands. Neumann and Wittgenstein[91] believe that it is likewise shared by the tissues of certain other organs, particularly the liver, spleen and ovary, whereas the lungs and blood are inactive.

Trudeau and Krause[92] attempted to treat some guinea pigs preventively with filtered emulsions of homologous lymph nodes, and other guinea pigs with emulsions of human glands which were tuberculous but non-caseous. In neither of the two series of experiments did the animals show any definitely marked resistance to the test infection.

Fontès[93] macerated definite proportions of bacilli in emulsions of tuberculous glands and, in like manner, other bacilli in emulsions of normal glands. After having left them in the incubator, he counted the bacteria at various intervals up to 120 hours and recovered fewer in the emulsion of tuberculous glands than in that of the normal glands. But I have been able to ascertain that this is a deceptive appearance resulting from the fact that the gland juice of tuberculous animals agglutinates the bacilli, while the normal gland juice does not.

13. Method of Bruschettini

Bruschettini[94] has studied, in guinea pigs and rabbits, a vaccination procedure which he uses in treating animals with a view to obtaining a serum for the treatment of human tuberculosis.

This vaccine is prepared in the following manner:

Virulent bacilli, from a culture on potato, are carefully suspended with quartz powder and chloroform, filtered through cotton, maintained for 12 to 18 hours on a water bath at 40°C., collected upon a filter, dried rapidly, taken up in suspension in physiological salt solution, then injected into the pleura of rabbits which have pre-

[90] Wien. klin. Wchnschr., 1909, **22**, 117.
[91] Ibid., 1906, **19**, 858.
[92] J. Med. Research, 1910, **22**, 277.
[93] Centralbl. f. Bakt., 1909. **50**, 7
[94] Ibid., 1913, **68**, 337.

viously received an injection of aleuronat or of Mellin's food (banana flour). At the end of 12 hours a new dose of aleuronat is injected, and after another 12 hours the animal is sacrificed. The exudate, collected aseptically, ground for a long time with quartz powder and physiological salt solution, decanted in order to eliminate the quartz, and finally supplemented with a few drops of chloroform, is left for 24 hours at 37°C. and *then centrifugated.* The product obtained, after control of its bacterial purity, is injected in doses of 1 cc. subcutaneously or of 0.1 cc. intravenously.

Animals thus treated preventively are more resistant to the test injection than are the controls, according to Bruschettini.

14. Attempts at vaccination by the inoculation of increasing doses of virulent bacilli

Following investigations as to the immunization of animals by repeated injections of very weak doses of virulent bacteria, Gerald Webb and W. Williams[95] had shown that rabbits and guinea pigs could be vaccinated against anthrax and even against tuberculosis by means of this procedure. For 9 months these authors kept alive a guinea pig into which they had injected progressively, beginning with a few cells, a total of 141,000 tubercle bacilli. In this animal, previously tested with tuberculin with negative result, they found no trace of tuberculous lesions. Gilbert and Forster, and later Lieb,[96] had the same result with 2 monkeys and with rabbits.

I had this method of vaccination studied by L. Bruyant,[97] altering the conditions of the experiment with a view to seeing whether it is preferable to inoculate a very small number of bacteria, 4 to 10 for example, several times and over a relatively long period, or whether it would better to begin with a few cells and progressively increase the doses. We were able to satisfy ourselves that, in both cases, with the very virulent strain used, it was impossible to render the animals capable of eliminating or resorbing their bacilli. The latter in successive doses, no matter how small, accumulate in the body and there set up, not an acute infection but lesions of resistance characterized by sclero-fatty hypertrophic degeneration of the liver and spleen, without distinct nodules.

[95] J. Med. Research, 1909, **20**, 1; 1911, **24**, 1.
[96] Ibid., 1910, **22**, 75.
[97] Compt. rend. Soc. de biol., 1911, **71**, 143.

But if one limits oneself, as we did later, to injecting into a guinea pig, once or twice, with a few weeks interval, 4 bacilli at the most (that is to say *one ten millionth of a milligram* of bacilli, weighed fresh and diluted in 1 cc. of physiological salt solution), it is found that the animals do not present any glandular swelling in the neighborhood of the point of inoculation and that they remain apparently in perfect health. If they are killed after one year or eighteen months, certain of them show very discrete tuberculous lesions incapable of hindering the regular functioning of the organs. They are generally localized in the liver or mediastinal lymph nodes. These lesions are often encysted in a shell of fibrous tissue and are altogether similar to those encountered in children, who, having been infected with a benign tuberculosis, die in the course of an intercurrent disease. They represent almost exactly the latent bacillary infection whose importance we know from the point of view of its protective influence against reinfections. Analogous researches published more recently by Lawrason Brown, F. H. Heise and S. A. Petroff[98] arrive at the same conclusion.

It would evidently be dangerous to use virulent human or bovine bacilli, even in a dosage of but a few organisms, with a view to artificially generating this state of resistance to reinfection, or of intolerance to natural inoculation, since the subjects, thus *pseudo-vaccinated,* remain in reality infected. They continue constantly under the menace of an extension of their foci, or of the possibility of their infection becoming generalized following upon the gradual caseation of a tuberculous follicle.

This consideration should be enough to cause the discarding of the idea of accomplishing antituberculous vaccination by direct inoculation, no matter how sparingly, of bacilli which are alive and virulent and therefore capable of setting up tuberculous lesions.

15. Attempts at immunization by the digestive tract

With C. Guérin[99] I published our experiments upon the mechanism of tuberculous infection, in which it was brought out that animals easily contract tuberculosis by the intestinal path, not only when

[98] J. Med. Research, 1914, **30**, 475.

[99] Ann. de l'Inst. Pasteur, 1905, **19**, 601; 1906, **20**, 353; 609:—Compt. rend. Acad. des sci., 1906, **142**, 1319.

very young, as von Behring said, but also in adult life, without the bacilli leaving any visible lesions after them in their passage through the wall of the digestive tract. In the course of the work we observed that if a calf is fed a certain quantity of human bacilli in two vaccinating meals (respectively 0.05 gm. and 0.25 gm.), with 45 days intervening, this animal may, after one year at least, be given one or several infecting meals of bovine bacilli without harm.

The resistance thus conferred by ingestion of the virus is more marked than that of cattle bovo-vaccinated intravenously by the method of von Behring, when tested against natural contamination through being stabled with animals bearing open lesions.

Roux and Vallée (at Alfort) have made the same observation.

This principle being established, in order that vaccination by the digestive tract might be realized practically, it became necessary to find a substitute for the virulent tubercle-producing (*tuberculigène*) human or bovine bacillus. This bacillus should be virulent but not tubercle-producing (*non tuberculigène*), should be readily tolerated by the lymphatic tissues and yet escape destruction by the phagocytic cells, at least for a considerable time; for other experiments had already given proof that resistance to *superadded* tuberculous infection persists only so long as there remain in the body a few bacilli or some lesion derived from the initial infection.

After that we turned to bovine bacilli heated for 5 minutes at 70°C. By feeding such bacilli in proper doses, we succeeded in conferring upon a few calves an appreciable resistance to virulent infections; but the results were found to be inconstant.

While we were carrying on these experiments S. Arloing was also trying to vaccinate cattle by the digestive tract with his homogeneous bacillus (human bacillus of attenuated virulence), and Vallée, on the advice of Roux, was trying to utilize in the same way a tubercle bacillus of *equine origin* which was but slightly virulent for the guinea pig and avirulent for cattle. But the homogeneous bacillus and equine bacillus are capable of producing follicular lesions: they do not therefore fully answer the requirement.

S. Arloing had also undertaken to verify the effectiveness of a tubercle bacillus product proposed by v. Behring in 1905 for the preventive and curative treatment of tuberculosis in man and cattle.

This product, *Tulase-lactine*, is made up of bacilli killed with chloral hydrate and suspended in an alkaline solution of milk sugar.

When injected subcutaneously it produced abscesses which were now and then very large and which were cured slowly and with difficulty. v. Behring thought that he obtained better results by letting it be absorbed through the digestive tract.

Control experiments by S. Arloing led to negative results. Moreover v. Behring himself before long gave up the use of his *Tulase.*

But from the various attempts there is clear proof that *in young animals of the bovine species,* immunity may be obtained through the *ingestion of living virus vaccines* and that, against natural contamination through confinement with other animals just as against artificial infection, this mode of vaccination appears to offer appreciable advantages over the intravenous. This is especially true from the point of view of the duration of resistance.

However, this method cannot be employed practically until we can use, as *vaccine virus,* an organism which is assuredly *non-tubercle-producing,* if not *avirulent.* This is our object in researches at present under way. I shall limit myself to a few general statements regarding them.

16. Vaccination with bile-treated (biliés) bacilli, of Calmette and Guérin

Following some observations which Guérin[100] and I had made on the subject of the modifications undergone by tubercle bacilli from *cultures* in their passage through the digestive tract, we were led to the finding that the bacillus grows perfectly upon media with a basis of potato or agar, saturated with pure bile glycerinated to 5 per cent. Furthermore after a certain number of successive replantings upon this medium, the bacillus acquires quite special physiological characters. The cultures greatly resemble those of the bacillus of glanders in appearance and their virulence diminishes progressively to such a point that, after about 70 replantings upon bile media, a calf will tolerate very well the injection of 100 mgms., whereas 3 mgms. of the same strain of bacilli, maintained in a parallel manner upon ordinary glycerin potato, produces in animals of the same age a fatal acute miliary tuberculosis within 28 to 35 days.

The only effect of this massive infection is to cause *a general disease which is like a typhoid fever* and which cures itself spontaneously after 15 to 20 days of fever, *without producing the slightest tubercle*

[100] Compt. rend. Acad. des sci., 1908, **147**, 1456; 1909, **149**, 716.—Ann. de l'Inst. Pasteur, 1911, **25**, 625; 1913, **27**, 162; 1914, **28**, 329.

formation,—as proved by subsequent autopsy of the animals. But at the same time it induces the formation of an abundance of antibodies and agglutinins which can be demonstrated in the serum.

Calves which receive 2 doses, one month apart, of 5 to 20 mgms. of biliated bacilli can be submitted, after still another month, to the test inoculation,—always intravenous,—of 3 mgms. of virulent bacilli, without manifesting the least malaise. They remain perfectly well. We have kept some of them longer than 18 months without its ever being possible to discover at autopsy the smallest tubercle in the lungs, abdominal viscera or different gland groups. The test bacilli nevertheless remain captive in the lymphatic system, although in a state of latency, as shown by the fact that if a number of guinea pigs are inoculated with ground up tracheal or mediastinal glands of a bullock vaccinated 18 months earlier, for example, a small but still fairly important proportion of the guinea pigs acquire tuberculosis. The test bacilli therefore remained all this long time in the animal's organs without manifesting their presence by producing nodules. And in the meantime they had lost neither their vitality nor their virulence, since on being carried over to a normal and susceptible animal, they produced in the latter a well defined tuberculosis (*fig. 31*).

It cannot be assumed that, if the glands of the vaccinated animals are virulent, they owe this virulence to the biliated organisms used to vaccinate, since the latter bacilli,—although capable of killing the guinea pig in a relatively small dose (1 mgm. intraperitoneally),—do not induce the formation of tubercles in this animal, any more than in the rabbit or monkey.

Since 1913 we have been studying, upon a series of cattle, the delayed effects of this method of vaccination from the point of view of the intensity and duration of resistance to natural contamination by a prolonged confinement with adult animals which have open lesions. To this end we arranged a stable so that one row of young cattle can be placed side by side behind a row of tuberculous animals which are abundantly discharging bacilli in their dejections. There is always a vaccinated animal on either side of a normal control, and all are fed from a common trough.

Our experiments were unfortunately interrupted by the war, but it appears, in so far as we are at present able to judge, that the use of our biliated bacillus is certainly without harm and that, if its efficacy

as a vaccine-virus asserts itself, prophylaxis against tuberculosis in cattle would be possible and practical. From the point of view of manipulation, it is devoid of danger, as it is with regard to a later elimination in the dejections or in milk, inasmuch as it has lost all capacity for forming tubercles. We have convinced ourselves that man will safely tolerate the injection of one one-hundredth of a milligram *intravenously* and meanwhile this bacillus has retained the property,—which is essential for the production of antituberculous immunity—of being able, as a simple saprophyte, to live in sym-

FIG. 31. INOCULATION OF AN EMULSION VIRUS-VACCINE INTRAVENOUSLY INTO A CALF

This inoculation is made into the jugular vein, which is rendered prominent by gently constricting the base of the neck with a cord.

biosis with lymphatic cells, without altering them. Naturally, the use of this vaccine can pretend to nothing more than to produce in animals the same conditions in relation to superinfections as are presented by already tuberculous subjects. The sole advantage—assuredly not negligible—which it can procure to them is that of protection against the forms of tuberculosis which, through the progressive extension of their lesions and the resulting emaciation and

cachexia, are prejudicial to the economic interests of cattle raisers and to public health.

I am bold enough to add that the proposal of its future use for the vaccination of children seems not improbable. To this end we are preparing a bacillus of the human type which, after having been carried through a long series of growths upon media with a basis of human bile to begin with, and then of beef bile, has lost its faculty of producing tuberculosis in the guinea pig and monkey. *Tuberculous patients tolerate it in fairly large doses either intravenously or by ingestion and without harmful effects.* It remains to be found out whether we may hope that its vaccinating powers are sufficiently sure and lasting against the natural infections of family life where there are individuals with open tuberculous lesions. But the answer to this question can come only from a prolonged experiment on a large scale and it should be carried out upon anthropoid apes *in an environment absolutely protected from any contamination through man.*

With a view to accomplishing this experiment, I have proposed setting up a sort of "nursery" for monkeys in one of the islands of the Los archipelago, off the coast of Guinea, in French Western Africa.

The war which has just bled and ruined Europe so terribly prevents the realization of this idea for the moment.

But I am not without hope that it will be started again, since, after the madness which has driven so many of the so-called civilized nations to destroy one another, the works of reparative peace will more than ever impose upon benevolent men the duty of striving to safeguard the innumerable human lives cut down by tuberculosis before their time.

CHAPTER XLIII

ATTEMPTS AT CHEMOTHERAPY IN TUBERCULOSIS

It would seem that *chemotherapy,* which gave such brilliant results in the treatment of the spirilloses and trypanosomiases following Ehrlich's work on the organic arsenic compounds, and in the treatment of mycotic affections with the introduction of iodides, might perhaps afford an effective method of combating tuberculosis. Investigators therefore should be informed of the recent efforts along this line, and the more so because, in our opinion, certain valuable suggestions stand out and are to be derived from them.

It was at first thought that the bacillus might be destroyed *in vivo* by using antiseptics of sufficient bactericidal power which would not destroy the vitality of the cellular tissues in the diseased individual. In this hope, creosote, guaiacol, thymol, formic aldehyde, vapors of various aromatic substances, and even anilin dyes were tried, but always unsuccessfully. None of these substances, whether introduced into the blood stream by subcutaneous or intravenous injection or by inhalation with the inspired air, actually reached the bacilli in the tuberculous lesions, since they are either destroyed or decomposed or fixed in the tissues before reaching the lesions themselves.

Moreover, it is vain to hope to convey by the blood, lymph or air any of these substances, which do not possess a specific chemical affinity either for the bacillus or the tuberculous cell, to the lesion itself. We fail to recognize the essential fact that the tuberculous cell, which is no longer a normal cell but a new complex formed through the symbiosis of tubercle bacilli with the elements constituting the giant cell (just as the lichen is the product of the symbiosis of an alga and a fungus), lives in a sense independently of the body which serves as host. It is no longer linked up with the latter by any capillary vessels. It isolates itself more and more in proportion to the degree of caseation and calcification which it undergoes. It receives its sustenance by osmosis alone and only in the latter way

do the normal cellular elements surrounding the lesion become impregnated with the diffused toxic products.

It is impossible, therefore, to conceive a great enough effect upon the tuberculous cell by a chemical agent except on condition that the latter possesses sufficient stability to be borne without decomposition or modification into the protoplasm of the cells surrounding the tubercles, and furthermore that it possesses the power of penetrating by osmosis into the tuberculous cell itself, thus to act upon the protoplasm of this cell or upon that of the bacilli which the cell contains.

It is still possible that certain chemical bodies may have an indirect action upon the tubercles *by favoring the transformation into fibrous tissue of healthy cells about the tubercles.* In this way probably the calcium salts act, and perhaps the various salts of copper. Once this fibrous tissue becomes sufficiently dense to interrupt or prevent osmosis, the complete isolation of the tubercle leads to its own death and the degeneration of the protoplasm and bacteria contained within it. This process of healing is fortunately a very frequent one. It is the rule in cases of discrete tuberculous infection and is seen fairly often in the more susceptible animals, like the guinea pig, following the inoculation of small numbers of bacilli of low virulence.

Up to now, the attempts at chemotherapy have been rather haphazard, either by phthisiologists working with tuberculous patients or here and there by a few investigators using laboratory animals. We shall confine ourselves to those which are worth mention and further study.

A. CALCIUM SALTS

The frequent observation, in man and in cattle and even in the small laboratory animals, of the phenomenon of calcification of the tubercles on the one hand, and, on the other hand, of the fact noted by Albert Robin, Darembert, and Ed. Dehaussy, that tuberculous individuals in the course of their disease suffer important losses of calcium, particularly in the form of calcariuria, would naturally cause physicians to utilize the salts of calcium in the therapy of tuberculosis. Those clinicians who hastily seized upon the so-called method of "recalcification" of Ferrier, with all sorts of variations, and have been applying it over a long period have apparently met with some success. There is no doubt, however, that from an experimental standpoint the method is ineffective.

Rénon claims to have obtained favorable effects in patients with the tannate and albuminate of calcium, instead of the carbonate and phosphate, which are at times badly tolerated.

It has been proposed to treat pulmonary infection by intravenous injections of freshly prepared solutions of 3 per cent pure calcium chloride, filtered and sterile. One can inject 10 to 100 cc. intravenously and the injections are well borne if made slowly. According to J. Bruhl and L. Buc,[1] cases with cavity formation are particularly apt to be benefited.

More recently Coutière,[2] on the basis of the oft repeated observation that workers in lime kilns are seldom tuberculous, has proposed the inhalation of hot dry air full of dust of quick lime and carbon dioxid.

In England, Edward E. Prest[3] at the Sanatorium at Ayrshire, claims good results from the use of a so-called colloidal preparation of calcium marketed by the "Crookes Laboratories." It is administered hypodermically in doses of 0.5 to 1 cc. every five days. Some individuals are very sensitive, vomit the day following the injections and have a considerable elevation of temperature. Patients who are subject to hemoptysis do well on it, and also cases with glandular involvement. This form of therapy, however, is contraindicated in febrile cases.

B. SALTS OF GOLD, SILVER, AND BISMUTH

In 1890, in a communication before the International Congress of Medicine at Berlin, Robert Koch spoke of the bactericidal effects of gold salts, more particularly those of the *double cyanide of gold and potassium,* on cultures of the tubercle bacillus. As little as one millionth part of the cyanide is enough to either prevent or arrest the development of the germ. G. Rosenthal and, more recently, Stefan Pekanowitch attempted to profit by this fact and to treat patients with daily subcutaneous injections and even with intratracheal injections of 5 to 15 mgm. This quantity was usually badly tolerated and had no good effect. This is not surprising, since gold salts are very unstable, and the hope of making them penetrate into the tissue without undergoing decomposition is illusory.

[1] Compt. rend. Soc. de biol., 1913, **74**, 880.
[2] Bull. Acad. méd., 1921, **86**, 410.
[3] Brit. Med. J., 1922, i, 53.

Feldt, on the advice of Professor Spiess, tried to associate the gold salt with cantharidin deprived of its specific toxicity through a combination with a very basic group, ethylene-diamine, the latter to serve as vehicle in conveying the gold salt to the tuberculus foci via the blood stream. This idea had its inspiration in the fact already observed by Liebreich, that cantharidin produces local reactions about the tubercles. The reaction, however, is in no sense a specific one. Seventy guinea pigs were treated over a period of ten months with subcutaneous injections of 0.04 mgm., and 30 rabbits with intravenous injections of 0.002 mgm. of this substance. The rabbits thus treated lived several months longer than the controls, but the course of the tuberculosis in the guinea pigs was not modified. I found that it is not difficult to accustom tubercle bacilli to growing in culture media in the presence of progressively increasing quantities of gold salts, and my collaborator, Maurice Breton,[4] had the same experience in using a solution of colloidal gold (0.125 grams of gold per 100 cc.) which M. Fourneau of the Pasteur Institute was good enough to prepare for us. Guinea pigs infected with tuberculosis and later treated by subcutaneous injections of this colloidal gold died after the same intervals as the controls.

Salts of silver and of mercury, although very bactericidal for the bacilli *in vitro,* are useless *in vivo,* and for the same reasons as the gold salts. Lydia M. De Witt[5] and Hope Sherman claim, however, that they have obtained some favorable results in a series of guinea pigs treated with a double salt of methylene blue and mercuric chloride.

Before the war, in which he lost his life, B. Sauton[6] had begun experiments at the Pasteur Institute on the chemotherapy of tuberculosis with salts of bismuth. He observed that bismuth in a concentration of 1 to 150,000 sufficed to arrest the development of cultures and that certain of its relatively non-toxic salts are sufficiently stable to be introduced into the body of animals without being fixed immediately in the tissue. These experiments would seem to merit further consideration.

C. SALTS OF COPPER

It was long ago noted that tuberculosis is rare among workers in the various copper industries (coppersmiths, in the manufactories of

[4] Compt. rend. Soc. de biol., 1913, **74**, 1200.
[5] J. Infect. Dis., 1921, **28**, 150.
[6] Compt. rend. Soc. de biol., 1913, **74**, 66.

copper acetate, verdigris, etc.). As early as 1894, both of the Lutons,[7] father and son, proposed the use of copper salts in the treatment of tuberculosis. E. Meissen[8] later demonstrated their lack of toxicity in animals.

On the other hand, this metal, as was shown by V. Nogeli, is extremely toxic for certain algae, even in concentrations of 1 to 1,000,000. At the same time it is useful against mycoses of plants and also against favus.

In culture media to which soluble salts of copper are added to concentrations of 1 to 100,000 and even to 1 to 1,000,000, the bacillus refuses to grow (Lydia M. De Witt and Hope Sherman)[9] but in the body these soluble salts are immediately transformed into insoluble salts and are fixed in the tissue about the point of inoculation, where they cause ulceration and necrosis.

Several investigators (H. J. Corper,[10] Stefan Pekanowitch) have treated guinea pigs and even tuberculous patients with intramuscular injections of the sulphate, acetate, oleate and albuminate of copper. In the case of the guinea pig, a dose of 1 mgm. per day is well tolerated. The copper fixes itself in the viscera and is not found either in the glands or in tuberculous pus, and the evolution of the disease does not appear to be favorably influenced.

V. Grysez[11] had no better results with repeated inhalations of verdigris, and R. Dalimier likewise was unsuccessful in treating animals with injections of the cyanide and sulphocyanide of copper. von Linden, at the advice of Professor Finkler, undertook at Bonn a series of experiments with cupric chloride, pure, and combined with methylene blue. In 1913, I happened to see the guinea pigs which she had first inoculated with human tubercle bacilli and afterward injected subcutaneously for several weeks with cupric chloride. There is no question but that in these animals the lesions were entirely different from those in the controls. The visceral organs contained nothing but fibrous cicatrical lesions, no caseous tubercles being present. von Linden states that she has observed the same effects with frequent inunctions of an ointment containing a com-

[7] Rev. de la tuberc., 1894, **2**, 83.

[8] Ztschr. f. Tuberk., 1913, **21**, 5.

[9] J. Infect. Dis., 1916 **18**, 368.

[10] Ibid., 1914, **15**, 51.

[11] Compt. rend. Soc. de biol., 1911, **70**, 780.

bination of copper and lecithin. Analyses are said to have proved that 65 per cent of the copper was retained in the tissues.

A. Strauss,[12] and later H. Eggers,[13] successfully treated some cases of lupus with this same combination of copper and lecithin (*Kupferlecithine*). They gave it intramuscularly, intravenously and by inunction and from photographs published by these authors it appears indeed that certain patients were very much benefited.

At the Sanatorium at Hohenhonnef, Meissen[14] injected tuberculous patients once or twice every week over a period of three months with one per cent solutions of chloride of copper and cupro-potassic tartrate. In 80 per cent of the cases he observed a decline of fever, a disappearance of bacilli from the sputum, and a manifest improvement in the general condition, without focal reactions. In his opinion this form of therapy merits thorough study.

Gensaburo Koga[15] made use of a solution of 0.2 per cent of cyanide of potassium and copper to which was added one per cent of chloride of calcium. This preparation, called *cyanocuprol,* is said to cause in the guinea pig the formation of connective tissue about the tubercles and a cicatrization of the latter. Sixty-three tuberculous patients in different stages were treated with progressively increasing doses, beginning with 0.01 gram intravenously; twenty-five are said to have been cured. According to Holland and Gaté,[16] this substance causes first a mononucleosis, with a destruction of red cells, and later a polynucleosis. In small doses it has no effect upon the bacilli, but after prolonged use in the guinea pig there is observed a falling of the hair and claws, and at times a dry gangrene of the extremities. Its sclerosing action seems manifest.

D. RARE EARTHS OF THE CERIC GROUP

The action of the salts of the rare earths of the ceric group (neodymium, praseodymium, samarium, lanthanum) upon the tubercle bacillus *in vitro* and upon experimental tuberculosis in laboratory animals has been studied by Frouin.[17] When added to culture

[12] München. med. Wchnschr., 1912, **59**, 2718.
[13] Beitr. z. Kl. d. Tuberk., 1913, **29**, 261.
[14] Ztschr. f. Tuberk., 1913, **21**, 410.
[15] J. Exper. Med., 1916, **24**, 107.
[16] Compt. rend. Soc. de biol., 1920, **83**, 178.
[17] Ibid., 1920, **83**, 756;—Compt. rend. de l'Acad. des sci., 1920, **170**, 1471.

media the growth of the human type of bacillus is inhibited; at a concentration of 0.05 per cent growth is retarded, with 0.1 per cent growth is completely arrested. When added in very minute amounts these salts cause the bacilli to assume a granular appearance. The content of the bacilli in fatty materials is decreased, the percentage being reduced from 38 or 40 per cent to 20 per cent, or even to 16 per cent. With normal animals the intravenous injections of solutions of these salts lead, by daily doses of 1 to 2 mgm. per kilogram, to a mononucleosis, with a very considerable polynucleosis, and an accompanying increase in weight. In tuberculous animals such injections cause the animals to survive two to three months beyond the controls, and there is a sclerosis of the lesions.

Numerous attempts have been made to use salts of the rare earths in the therapy of human tuberculosis, chiefly by Rénon,[18] Grenet and Drouin,[19] Esnault and Brou,[20] Pissavy, and Hudelo. In incipient tuberculosis the results have been somewhat favorable, as in cutaneous tuberculosis also. In advanced tuberculosis the results have been entirely negative, and in febrile pulmonary tuberculosis the therapy has been of questionable value. Usually the compounds are employed by intravenous injection, daily doses being given throughout a series of twenty consecutive days, and these courses of treatment are repeated several times. The dose is from 0.08 to 0.1 gram of the sulfate. It would appear that prolonged treatment sometimes leads to sclerosis, but in experimental tuberculosis of animals no benefit seems to result.

E. RADIOACTIVE SUBSTANCES

These substances are without influence upon cultures of the tubercle bacillus. Rénon has studied experimentally the bromide and the sulfate of radium, using amounts of from 5 to 10 millionths of a milligram. When treated on the day following the inoculation of virulent organisms the guinea pigs survive some fifteen to twenty-five days longer than the controls, but all attempts at therapy in patients have failed to result in any marked improvement. The results with the salts of thorium have been equally discouraging.

[18] Bull. et mém. Soc. méd. d. hôp. de Par., 1920, **44**, 602; 1425.
[19] Ibid., 1920, **44**, 589.
[20] Ibid., 1920, **44**, 606.

F. ARSENICAL COMPOUNDS

Arsenical compounds, such as sodium arsenite, sodium cacodylate, atoxyl, arsacetine, and neosalvarsan, may, according to Aaron Arkin and H. J. Corper,[21] react as a stimulant of the general metabolism, and in this way be of decided benefit to tuberculous patients who show a positive Wassermann reaction and in whom the evolution of the tuberculosis is aggravated by an hereditary syphilis or by a recently acquired process. Such compounds have no effect upon the tubercle bacilli however.

G. BENZYL ALCOHOL, XYLOL, CREOSOTE SERIES

That benzyl alcohol dissolves tubercle bacilli and that daily subcutaneous injections of 2 cc. are well tolerated by tuberculous guinea pigs has been shown by J. Jacobson. Xylol has been utilized by Volpino, who treated guinea pigs, beginning four days after infection with tuberculous sputum, by the subcutaneous injection of 0.5 cc. repeated every two to four days, or by daily injections of 0.5 to 1 cc. during a period of two weeks. The animals withstood the injections well, since they were but slightly toxic, and phagocytosis was stimulated.

Other derivatives of benzol, such as cumene, possess analogous properties and act more particularly upon the tuberculous foci. Volpino hopes to secure interesting results with patients by the subcutaneous administration of these hydrocarbons diluted to 10 per cent in oil.

Inhalations of anhydrous vapors of different antiseptics (creosote, guaiacol, turpentine, etc.) have been used for a long time by many investigators and clinicians. Charles Richet, P. Brodin and F. Saint-Girons have utilized these substances dissolved in oil or vaseline and in a volatilized state, alternating the applications so that patients did not inhale the same vapor twice in succession. It was observed, after several inhalations, each lasting two hours, that there was a manifest improvement in the general condition of the severely ill patients. The appetite improved, the weight increased, and the expectoration and the cough decreased.

In the treatment of experimental tuberculosis of guinea pigs substances of the creosote series (resorcine, thymol, para-, ortho-, and

[21] J. Infect. Dis., 1916, **18**, 335.

meta-cresols, guaiacol, hydroquinine, cacodylate of guaiacol) failed to give favorable results (L. M. DeWitt, B. Suyenaga and H. G. Wells[22]).

II. IODINE AND IODINE COMPOUNDS

The very manifest curative effects of iodine and iodides in the mycoses have led investigators to attempt similar medication in tubercular infection. Arnold Cantani[23] in 1909, and Copelli[24] in the same year, had noted that the addition of iodine to tuberculin suppressed the thermic reaction produced by the latter in tuberculous guinea pigs, and that, under the influence of the iodine, a marked increase in the number of the leucocytes was observed with a rapid increase in the opsonic index. On the other hand, G. Kapsenberg[25] treated tuberculous guinea pigs and rabbits with mixtures of iodine and bacillary extract, claiming to have arrested the progress of the disease. Von Linden, using a combination of iodine with methylene blue secured the same survival period in tuberculous guinea pigs, and the same evidences of sclerosis as with copper chloride. Patients with lupus or with the so-called surgical tuberculoses bear equally well, according to Strauss, repeated inoculations of this product. It seems that treatment by iodine, either by subcutaneous or intravenous injections in the form of iodoform in ethereal solution (D. Rothschild[26]) or by daily ingestions of 40 to 150 drops of tincture of iodine, as has been proposed by Louis Boudreau[27] in France and later by Therasse and Henno[28] in Belgium, augments the mononucleosis and diminishes the number of lymphocytes, favorably affecting many cases of pulmonary tuberculosis. L. Boudreau administered the tincture of iodine, diluted in the drink, in doses at first very weak, then progressively increasing up to the extreme limit of tolerance. With certain patients an intolerance was very quickly shown. Tuberculosis of the glands responded best to this treatment.

H. Dufour[29] preferred to give the iodine by intravenous injection

[22] J. Infect. Dis., 1920, **27**, 115.
[23] Ztschr. f. Hyg., 1909, **62**, 34.
[24] Boll. d. Soc. med. di Parma, 1909, 2. s., ii, 141.
[25] Berl. klin. Wchnschr., 1913, **50**, 879.
[26] Deut. med. Wchnschr., 1913, **39**, 404.
[27] J. de méd. de Bordeaux, 1914, **44**, 3.
[28] Scalpel, 1921, April 9.
[29] Bull. et mém. Soc. méd. d. hôp. de Par., 1920, **44**, 693; 695.

in the form of iodaseptine (an iodo-benzo-methyl-methane compound) in doses of 2.5 to 5 cc. quantities, repeated daily throughout twenty days, followed after an interval of two weeks by a second series of injections of the same character. Other clinicians have employed an electro-chemically prepared colloidal iodine, a preparation which is painless upon injection, and which does not produce a thermic reaction. Georges Petit has treated 260 cases of tuberculosis with this substance and states that he has secured 128 definite cures.

I. DYESTUFFS

It was but natural to attempt the chemotherapy of tuberculosis with stains which react with fatty substances or dyes which are soluble in fats. This has been done by Hope Sherman,[30] H. J. Corper,[31] Lydia M. DeWitt,[32] and Paul A. Lewis,[33] with aqueous solutions or solutions in 70 per cent alcohol or oil, of Sudan III, Scarlet R, nile blue (sulfate), of indophenol-blue, of dimethylamido-azobenzol, of induline, of Bismarck brown, etc. But these dyes do not diffuse throughout the tissues, remaining localized at the point of inoculation. It is the same with dyestuffs of the benzopurpurine series (trypan red and trypan blue of Ehrlich) with which the staining action extends to the tubercles. But the vitality of the bacilli is not changed in the least. As with all the aniline dyes, even those which stain the bacilli with the greatest intensity, the virulence is neither modified by *in vitro* contact nor do they have any influence upon the development of the infectious process in experimental animals.

K. FATTY ACIDS OF CHAULMOOGRA OIL AND OF COD-LIVER OIL

The encouraging results obtained with chaulmoogra oil in leprosy led Leonard Rogers[34] to determine the effect of the sodium salts of the fatty acids of this oil, and of cod-liver oil as well, in tuberculosis. The fatty acids of chaulmoogra oil are hydnocarpic acid (melting point 59 to 61°) and chaulmoogric acid (melting point 68°). They are found in *Tarakogenos kurzii* and in three species of *Hydnocarpus*.

[30] J. Infect. Dis., 1913, **12**, 249.
[31] Ibid., 1913, **12**, 274.
[32] Ibid., 1914, **14**, 498.
[33] J. Exper. Med., 1917, **25**, 441.
[34] Brit. Med. J., 1916, ii, 550;—Indian Med. Gaz., 1919, **54**, 165; 218; 1920, **55**, 125;—Lancet, 1921, i, 1178.

Rogers has confined his experiments to the sodium salts (sodium hydnocarpate) prepared from the oil of *Hydnocarpus wightiana*, injecting the material either subcutaneously or intravenously. When injected under the skin the treatment is free from pain and the action is slow with the weak doses employed, 0.2 cc. of a 3 per cent solution. Intravenous injections act more rapidly, at least in leprosy. The injections are given weekly in amounts of from 1 to 5 cc. but they produce violent thermic reactions which diminish the resistance of the patients. *In vitro*, the fatty acids of chaulmoogra oil, and also those of cod-liver oil, linseed oil, sardine oil, and soja oil, possess the property of altering the fatty-waxy ectoplasm of acid-fast bacilli. The tests made in India by Rogers, and by other physicians, with sodium morrhuate have not been carried very far. Of 6 cases treated, 5 showed a permanent amelioration of their condition, still definite after a year. Cure has been obtained in 17 cases of tuberculosis of the glands of the neck. In but one case was there a recedive.

In tuberculosis it is essential to proceed with more care than in leprosy, for the reactions are more lively. They are accompanied by fever, cough, abundant expectoration, and sometimes hemoptyses. According to Rogers, they are the result of an intense destruction of bacilli, but in spite of all, the patient recovers from them rapidly, gains in weight, the sputum diminishes in amount, the temperature falls, and improvement results. Walker and Sweeney[35] have studied the bactericidal power of oils or of fatty acids on different species of acid-fast bacilli. The action may be intense, and, with the chaulmoogra series still manifests itself in dilutions up to a million. But with the cod-liver oil acids it does not occur at all, so that apparently the derivatives of the chaulmoogra oil alone can be efficacious in the therapy of tuberculosis. Unfortunately the recent work of Max Biesenthal,[36] and then that of Kolmer, Davis and Jager[37] destroys this hope. Experimentation upon tuberculous guinea pigs shows that injections of these products do not exercise any favorable action upon the development of the disease.

It must be recognized that up to the present, despite the great number of attempts made to discover among chemical agents a substance capable of arresting the development of experimental

[35] J. Infect. Dis., 1920, **26**, 238.
[36] Am. Rev. Tuberc., 1920, **4**, 781.
[37] J. Infect. Dis., 1921, **28**, 265.

tuberculosis in the guinea pig and the rabbit, these efforts have been in vain. But this is not a reason for discouragement. Certain attempts among those which we have cited deserve further attention. It will be desirable, for example, to give further consideration to those with iodized compounds, which, if they do not appear full of promise, give, nevertheless, some definitely favorable results.

We must always remember that it is useless, perhaps it may be dangerous, to inject at random, as has been too often done, such and such a chemical into patients with the vague hope of discovering a specific activity. This is a practise which should be condemned. Experimentation alone, methodically conducted upon animals sensitive to tuberculosis, will enable us to explore with profit the immense perspectives that chemotherapy offers.

CHAPTER XLIV

SCIENTIFIC PRINCIPLES WHICH SHOULD SERVE AS A BASIS FOR PROPHYLAXIS AGAINST TUBERCULOSIS

Among the "new facts" which, during recent years, have considerably added to our knowledge of tuberculosis, attention should be called to those which should in future dominate the whole prophylaxis of this disease.

They may be summed up in the three following propositions:

1. *Infection with the tubercle bacillus is abundantly distributed and carried by civilization throughout the world. In the immense majority of subjects susceptible to tuberculous infection (men and cattle principally), it is compatible with the appearance of health. For their organism the tubercle bacillus most frequently remains an inoffensive parasite.*

2. *It is only the massive infections with the tubercle bacillus, occurring in the young or in adults free from all previous infection, which determine from the beginning a generalized or localized disease of the lymphatic system.* The most common types of it are:

a. Acute miliary tuberculosis, almost always rapidly fatal;

b. Tubercle bacillus septicemia, the severity of which is dependent upon the source, virulence and number of the infecting bacteria. It often passes unnoticed so mild is it, particularly in young persons, and then ends in occult bacillary infection (*without tuberculous follicles*), or in the latent tuberculization of one or several lymphatic glands. Or again, after having assumed the appearance of an inflammatory disease like typhoid fever (*typho-bacillose*), it becomes localized in a gland group and there sets up progressive tuberculous lesions which later become abundantly seeded in other organs,—especially in the lungs. Thus tubercle bacillus infection, contracted in early life, may bring about, *more or less tardily*, diverse chronic forms of tuberculosis and phthisis.

3. *The benign tubercle bacillus infections, which remain occult or latent for years, induce, in those who harbor them, a peculiar state of resistance to new infections. When the latter are superadded they call*

forth, according as they are more or less abundant, virulent and near together, a special phenomenon of intolerance for the tubercle bacillus (which we have studied under the name of the phenomenon of Koch). The infected organism then tends to rid itself of its bacilli by forming abscesses which caseate more and more quickly and intensely, thus producing purulent softening of the tissues (cavities). The forms of tuberculosis which result develop slowly as a rule. They react in a variety of ways upon the general condition of the individual, but present the gravest danger from the standpoint of the scattering of infectious elements in the environment.

Travel and commercial transactions, the ever denser accumulation of peoples in cities, the crowding of families into dwellings too small, badly aired and with too little sun, and principally the ignorance which prevents avoidance of opportunities for contagion or superinfection are assuredly the principal causes of the extreme diffusion of tuberculosis among all peoples. But the essential factors of contamination are the disseminators of virulent germs. And it should be added that the latter are not exclusively the spitting phthisical cases and those with open tuberculosis, as was formerly thought; they include also the occult or latent tuberculous, who, well themselves, almost always ignorant of their reacting to tuberculin and therefore not at all suspecting that they may be the source of a disease, *eliminate bacilli intermittently with their glandular secretions or with their dejections.*

These *disseminators of bacilli and the bacilli which they scatter* are so numerous that it is no wonder that in the cities the children up to 5 years are already contaminated in a proportion of 55 per cent and that beyond 15 years, scarcely 5 per cent of the total population has succeeded in completely escaping infection!

The tubercle bacillus however does not exist everywhere; it is not "ubiquitous," as is too often incorrectly stated. It is found only where deposited by man or animals who harbor it. And we know that when only a small number of virulent organisms enter by accident into an organism entirely free of infection, there results in the immense majority of cases, only a benign infection which may remain indefinitely hidden or latent and which is revealed only by tuberculin reactions.

The real and the serious danger therefore, as regards the organism entirely free from tuberculous infection, lies in massive inoculation.

For the bearers of latent infection it lies in the repeated super-infections which develop their intolerance to the bacilli and aggravate their lesions through the increasing intensity with which the phenomenon of Koch produces itself.

A. THE TUBERCULIZABLE SOIL (TERRAIN TUBERCULISABLE)

For the future it is an evident truth that *tuberculous infection is brought about only by the bacillus, and that the severity of the infection is above all dependent upon the number, quality and source of the infecting elements, as well as upon the paths chosen by the latter for entering into the organism.* But we should not fail to recognize that the defensive reactions set up by this organism differ with the individual. Age, integrity of the lymphatic organs, healthy or pathological condition of the heart, of the vessels, lungs, liver, kidneys, skin, etc., variations of nutritional and respiratory exchange, intervene in each individual under special conditions which hinder or favor the defensive reactions.

Each one battles against the infection with his natural weapons, which are his leucocytes, his lymphatic glands, his cellular ferments, and his hereditary or acquired qualities of resistance or intolerance to the bacillus. And these natural weapons are never adapted in just the same way to the same defensive functions in any two individuals similarly exposed.

Thus must be understood the rôle of what French clinicians call the "terrain tuberculisable." "In tuberculosis," said Pidoux in speaking of the work of Villemin, "it is the soil which is everything and not the seed!" The inverse of this proposition would be equally inaccurate. Let us be on our guard, in every sense, against exaggerations opposed to scientific truth.

Some of the great masters have ingeniously condensed into clean-cut maxims, made for the public rather than for physicians, certain notions which they regarded as specially in need of being propagated, for example:

Alcoholism makes the bed for tuberculosis (l'alcool fait le lit de la tuberculose—Landouzy); *Tuberculosis is contracted by too frequent visits to the bar* (tuberculose se prend sur le zinc—Hayem); *Tuberculosis is a disease of poverty and ignorance*—Landouzy), etc.

Personally I feel it would be better not to repeat these aphorisms too often to the general public, since they tend to distract attention

from the essential aim which we ought and wish to pursue, which is to *dry up the sources of infection or to render them harmless.*

Certain it is that tuberculosis finds infinitely fewer victims among the well-to-do and educated than among ignorant unfortunates, given up to alcoholism, badly fed and housed in airless, sunless tenements. But alcoholism, poverty, defective food supply, unsanitary housing, do not render a man tuberculous *where the bacillus does not exist.* They are only,—and this is already too much,—factors of bodily enfeeblement which paralyze or impede the action of the natural weapons of defense after the infection has been able to establish itself.

There is no doubt as to the fact that improvement of the material conditions of life, laws capable of developing provident institutions or obligatory insurance, of increasing the wages and well-being of laborers, of suppressing alcoholism, for improving sanitary conditions of cities and habitations, would contribute in a large measure to limiting the sources and *chances of contagion.* But legislators and economists are powerless to realize the necessary reforms until the people have been educated to the point of demanding them. At any rate they would be only a beginning of antituberculosis action. The latter can be really effective only by devoting its efforts to the *preservation* of individuals and communities *against infection* and above all *against the tuberculous reinfections* which engender the grave and contagious forms of tuberculosis.

From the strictly medical point of view the question of *tuberculizable soil* has remained rather obscure from the fact that certain clinicians still regard as *candidates for tuberculosis* those individuals who, on clinical examination, present what by agreement are called the characteristic "stigmata," inherited or acquired (*see Chapter XIX*): hereditary dystrophies, anatomical deformities of the thorax, chronic swellings of the glands, demineralization, signs of Grancher, etc.

Now these "candidates for tuberculosis" in reality harbor bacilli. *All of them react to tuberculin.* Those among them who are not exposed to frequent and massive reinfections acquire the state of immunity peculiar to the bearers of latent lesions. The others, who are only too often obliged to live in a more or less intimate contact with disseminators of bacilli (*semeurs de germes*), are all the more likely to become phthisical as their natural means of defense are the more reduced.

We see this very condition being produced in animals of the bovine species for example. If a certain number of calves, known to be free from tuberculosis through complete absence of reaction to tuberculin, are put into the same stable with adult cows which have open bacillary lesions, it is found that, after a few months, all of the calves without exception react to tuberculin. And if the infecting contact be prolonged, some of the calves develop more severe tuberculous lesions with tendency to progression, while in the majority the disease gives no clinical sign of its presence: it remains localized in one or several glands which can be detected only by autopsy, and then often with difficulty.

It may be said therefore that *all calves*, without exception, if they are still free from bacillary infection,—and the same applies to children under the same conditions,—offer *a tuberculizable soil.* In the bovine species just as in the human species there is no subject who is *not tuberculizable unless he is immunized*—as far as one can be to the tubercle bacillus—*by a previous benign infection.*

No matter how fertile a field, it will never grow grain other than that sown by laborer, birds or winds.

The truth is equally evident that no matter how *tuberculizable* an animal or human organism may be, it cannot be rendered tuberculous except through the *implantation* of bacilli introduced into it.

A child is never seen to contract tuberculosis, even though condemned to live in the most unhealthy tenement and in the most frightful conditions of poverty, nor a calf in the most unhealthy stable, unless virulent germs are introduced *intermittently* or *continuously* by sick people or sick animals.

Before all then and above all, our efforts at combating tuberculosis should be concentrated against introductions of infection and principally against the frequent and abundant ones.

B. HOW TO DRY UP THE SOURCES OF TUBERCULOUS INFECTION

We know now that subjects who react positively to tuberculin, although perfectly healthy in appearance, eliminate virulent tubercle bacilli *from time to time* and disseminate them in their secretions.

These sources of virus, manifest only at intervals, are all the more dangerous in that they are almost always unsuspected. They spread bovine tuberculosis in stable and pig sty. Through their agency, human tuberculosis infiltrates into parts of the world which,

by virtue of their isolation, should be best protected. On account of their *intermittent character*, it is and probably always will be very difficult to discover them, no matter how perfect may be our scientific means of investigation.

In the present state of our knowledge, *we should regard as suspicious every individual who gives a positive reaction to tuberculin, even though he be of healthy appearance.* Possibly this particular individual, human being or cattle, is not eliminating any bacilli and for weeks, months or years, is completely harmless. But suddenly, without any perceptible warning, the dejections or certain of the glandular secretions (milk particularly) may contain bacilli.

It is necessary therefore, *in environments not yet infected*, to constantly *safeguard* young children and young domesticated animals against this danger. To that end it is indispensable that these young children and young animals be prevented from consuming suspected milk which has not been heated for a sufficient time (at least 30 minutes at 70° or 15 minutes at 80°C.) to insure the destruction of virulent germs. And it is not less indispensable to avoid possible contamination of food by bacilli from excreta. Indeed this contamination is unfortunately very frequent. It occurs not only by means of dirty hands, soiled linen, vegetables and fruits, but also through *flies*[1] and by earth or dust from sewage fields.

Too many precautions cannot be taken against such materials. It is indeed well established that clean cities, provided with incinerators for their refuse and with a good sewer system complete with a biological purification plant,—this is the case in a fairly large number of English cities for example,—have a tuberculosis morbidity and mortality infinitely lower than cities whose departments of hygiene are inefficient.

There is no necessity for disguising the fact that it will be a long and very difficult task to dry up the "réservoirs of the virus," namely *those who expectorate bacilli. The expectoration of ill persons and cattle having open tuberculous lesions, are and will remain the most formidable sources of contagion, not only for young children* and *young*

[1] The rôle of the fly in the propagation of tuberculosis has been pointed out and studied by various authors, especially: Lord; (Publications of the Mass. Gen. Hosp., 1906, Feb.); Weber; (Philadelphia Med. J., 1906, Nov.); André; (Bull. Soc. méd. d. hôp. de Lyon, 1906, **5**, 321); and Jacob and Klopstock; (Tuberculosis, 1910, **9**, 496).

animals, but also for *adults*, since it is such open cases which usually cause the *massive* and *frequent* infections, of whose pernicious rôle in the genesis of *phthisis* we have already spoken.

Protection can be made efficient only by organizing a whole system of detection (*depistage*) based upon bacteriological examination and at the same time upon the judicious use of tuberculin reactions. And this detection is not to be effected in man or animals without great difficulty. It depends upon the insight and science of physicians and veterinarians; it depends also upon organizations for protection against tuberculosis and, still more, upon attention on the part of those most directly interested, either because they cannot escape living with patients or because they fear the economic consequences of the spread of tuberculosis on their farms.

Tuberculous infection is so very widespread, and so serious in certain groups, that it is almost impossible to visualize its control to begin with, and its extinction later, except by *vaccination* of all susceptible human beings and animals.

This vaccination can be accomplished, inasmuch as it occurs spontaneously in an immense number of individuals, in consequence of one or more mild infections contracted, most often, in early life.

It would certainly be dangerous to induce such infections artificially by the use of *virulent* tubercle bacilli. But we have seen that *it is possible to modify certain of their properties in such a way that the bacilli lose their faculty of producing the cellular changes characteristic of tubercles.* To continue the research along this path is the rôle of the investigator.

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